PB81-163867

Quantitative Ultratrace Transition Metal Analysis of High Salinity Waters Utilizing Chelating Resin Separation: Application to Energy-Related Environmental Samples

(U.S.) National Bureau of Standards
Washington, DC

Prepared for

Environmental Protection Agency
Washington, DC

Aug 79

U.S. Department of Commerce
National Technical Information Service
NTIS

NBS

U.S. Department of Commerce

National Bureau of Standards

Center for Analytical Chemistry Washington, DC 20234

EPA

United States Environmental Protection Agency

Office of Energy, Minerals, and Industry Washington DC 20460

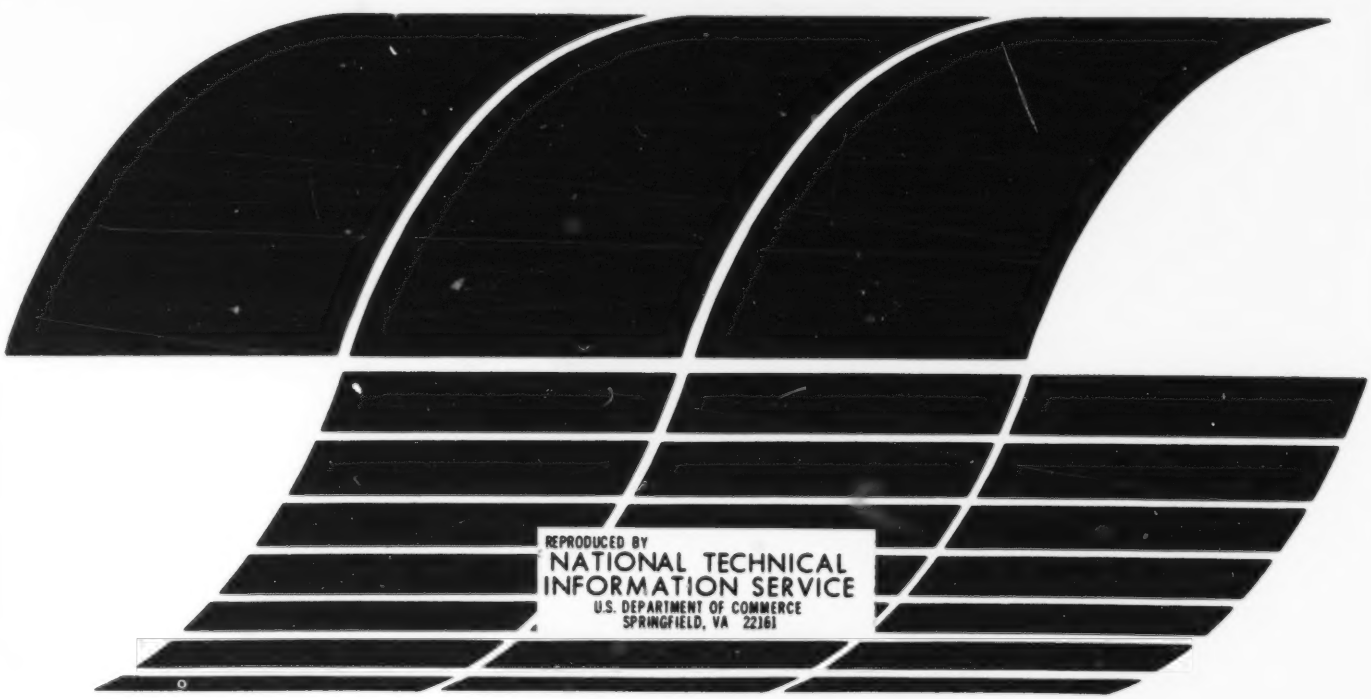
EPA-600/7-79-174
August 1979

PB81-163867

Research and Development

Quantitative Ultratrace Transition Metal Analysis of High Salinity Waters Utilizing Chelating Resin Separation

Interagency Energy/Environment R&D Program Report



REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the INTERAGENCY ENERGY-ENVIRONMENT RESEARCH AND DEVELOPMENT series. Reports in this series result from the effort funded under the 17-agency Federal Energy/Environment Research and Development Program. These studies relate to EPA's mission to protect the public health and welfare from adverse effects of pollutants associated with energy systems. The goal of the Program is to assure the rapid development of domestic energy supplies in an environmentally-compatible manner by providing the necessary environmental data and control technology. Investigations include analyses of the transport of energy-related pollutants and their health and ecological effects; assessments of, and development of, control technologies for energy systems; and integrated assessments of a wide range of energy-related environmental issues.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/7-79-174		PB81 163867

4. TITLE AND SUBTITLE
Quantitative Ultratrace Transition Metal Analysis of High Salinity Waters Utilizing Chelating Resin Separation: Application to Energy-related Environmental Samples

5. REPORT DATE
August 1979

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Howard M. Kingston

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Center for Analytical Chemistry
National Bureau of Standards
Washington, DC 20234

10. PROGRAM ELEMENT NO.
EHA-553

11. CONTRACT/GRANT NO.
1NE625C

12. SPONSORING AGENCY NAME AND ADDRESS
U. S. Environmental Protection Agency
Office of Research & Development
Office of Energy, Minerals & Industry
Washington, DC 20460

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/17

15. SUPPLEMENTARY NOTES
This project is part of the EPA-planned and coordinated Federal Interagency Energy/Environment R&D Program.

16. ABSTRACT

In order to accurately evaluate the impact of energy related activities, such as offshore drilling on the sea water, it is necessary to measure trace element concentrations in the presence of considerably higher levels of alkali and alkaline earth elements.

This report describes a technique which was developed for the elimination of the alkali and alkaline earth elements Na, K, Ca and Mg from the trace transition elements in sea water samples. This was accomplished by passing an ammonium acetate solution through a column of Chelex 100 resin after a sea water sample had been chelated. The alkali and alkaline earth elements were eluted from the column by ammonium acetate and the trace transition elements were then collected using nitric acid.

The quantitative concentration, separation and removal of selected transition elements was tested using radiochemical tracers. The study revealed >99.9 percent recovery of Cd, Cu, Mn, Ni and Zn, using a 100 mL sample, and >99 percent with a 1 liter volume. Cobalt and Pb exhibited >99 percent and >98 percent recoveries, respectively, from a 100 mL sample, and >97 percent from a 1 liter volume. Iron was found to be recovered only approximately 92 percent in either volume.

The concentration and separation technique was applied to Chesapeake Bay and Alaskan sea water samples. The samples were introduced into a graphite furnace and were analyzed by graphite furnace atomic absorption spectrometry. Analysis of concentrations below ng/mL for the trace elements mentioned was possible using this combination of sample preparation and instrumental analysis and no interelement interferences occurred. The combination of this sample preparation technique and the graphite furnace atomic absorption sensitivity enables extreme detection limits to be achieved for the elements mentioned, and gave measurements that were reliable and reproducible.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chelating Resin Sea Water Salt Water Separation Trace Metals Trace Element Analysis Electrothermal Atomization	Atomic Absorption Spectrometry Neutron Activation Analysis Spark Source Mass Spectrometry	Processes & Effects: Ecological Effects Fuel: Hydroelectric	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

☆U.S. GOVERNMENT PRINTING OFFICE: 1979-311-046/295

QUANTITATIVE ULTRATRACE TRANSITION METAL ANALYSIS OF HIGH SALINITY WATERS UTILIZING CHELATING RESIN SEPARATION: APPLICATION TO ENERGY-RELATED ENVIRONMENTAL SAMPLES

by

Howard M. Kingston
Center for Analytical Chemistry
National Bureau of Standards
Washington, DC 20234

Interagency Agreement No. EPA-IAG-D5-E684
Program Element No. EHA-553

EPA Project Officer: J. Stemmle

Environmental Protection Agency
Washington, DC 20460

This study was conducted
as part of the Federal
Interagency Energy/Environment
Research and Development Program

Prepared for

OFFICE OF ENERGY, MINERALS, AND INDUSTRY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DISCLAIMER

This report has been prepared and reviewed by the Center for Analytical Chemistry and the Office of Environmental Measurements, National Bureau of Standards, and reviewed by the U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency. In order to adequately describe materials and experimental procedures, it was occasionally necessary to identify commercial products by manufacturer's name or label. In no instance does such identification imply endorsement by the National Bureau of Standards or the U. S. Environmental Protection Agency nor does it imply that the particular products or equipment is necessarily the best available for that purpose.

FOREWORD

The role of the National Bureau of Standards (NBS) in the Interagency Energy/Environment R&D program, coordinated by the Office of Research and Development, U. S. Environmental Protection Agency, is to provide those services necessary to assure data quality in measurements being made by a wide variety of Federal, state, local, and private industry participants in the entire program. The work at NBS is under the direction of the Office of Environmental Measurements and is conducted in the Center for Analytical Chemistry, the Center for Radiation Research and the Center for Thermodynamics and Molecular Science. NBS activities are in the Characterization, Measurement, and Monitoring Program category and addresss data quality assurance needs in the areas of air and water measurement methods, standards, and instrumentation. NBS outputs in support of this program consist of the development and description of new or improved methods of measurement, studies of the feasibility of production of Standard Reference Materials for the calibration of both field and laboratory instruments, and the development of data on the physical and chemical properties of materials of environmental importance in energy production. This report is one of the Interagency Energy/Environment Research and Development Series reports prepared to provide detailed information on an NBS measurement method or standard development.

C. C. Gravatt

C. C. Gravatt, Chief
Office of Environmental Measurements
National Bureau of Standards

CONTENTS

Foreword iii
Abstract vi
Figures viii
Tables ix
Acknowledgment xi

1. Introduction 1
2. Summary and Conclusions 2
3. Literature Review 6
4. Experimental Procedures 12

Materials 12
Apparatus 13
Procedure 16

5. Results and Discussion 21

Separating Agent Selection 21

Concentration and Separation Parameters 33

Aspects of the Separation 36

Physical Parameters and Extension of the Separation of Larger Volumes 38

Quantitativeness of the Total Recovery of Selected Transition Elements Using Radiochemical Tracers 45

Evaluation of the Compatibility of the Chelex 100 Concentration and Separation with Spark Source Mass Spectrometry 50

Evaluation and Practical Use of the Chelex 100 Sample Preparation Method Utilizing Graphite Furnace Atomic Absorption Spectrometry Analysis 51

References 58
Appendices

A. The Abundances of the Chemical Elements in Sea Water (1) 62
B. The Selectivity Coefficients for Chelex 100 Resin Based on Zn^{+2} (26) 66
C. Typical Sub-Boiling Distilled Water Impurity Concentrations PPB by Wt (ng/g) (44) 67

CONTENTS

(Continued)

Appendices

D. The Experimental Conditions for Analysis of the 2.5 M HNO_3 Fraction Containing the Trace Metals Using A Perkin-Elmer Model 603 Atomic Absorption Spectrophotometer Equipped with a Heated Graphite Atomizer, HGA-2100 69

E. A List of Resin Volume for Chelex 100 in Different Ionic Forms Based on Na as 1.00 (58) 70

F. A Crystal Residue Observed in Frozen Neutral Sea Water Samples ... 71

ABSTRACT

In order to accurately evaluate the impact of energy related activities, such as offshore drilling, on the sea water system, it is necessary to measure trace element concentrations in the presence of considerably higher levels of alkali and alkaline earth elements.

This report describes a technique which was developed for the elimination of the alkali and alkaline earth elements Na, K, Ca and Mg from the trace transition elements in environmental samples. Sea water is an environmental sample where the alkali and alkaline earth elements are those of the highest concentrations while the trace transition elements are present in the lowest concentrations; it is an extreme example of this type of matrix. The separation was accomplished by passing an ammonium acetate solution through a column of Chelex 100 resin after a sea water sample had been chelated. The alkali and alkaline earth elements were eluted from the column by ammonium acetate and the trace transition elements were then collected using nitric acid.

The quantitative concentration, separation and removal of selected transition elements was tested using radiochemical tracers. The study revealed >99.9 percent recovery of Cd, Cu, Mn, Ni and Zn, using a 100 mL sample, and >99 percent with a 1 liter volume. Cobalt and Pb exhibited >99 percent and >98 percent recoveries, respectively, from a 100 mL sample, and >97 percent from a 1 liter volume. Iron was found to be recovered only approximately 92 percent in either volume.

The concentration and separation technique was applied to Chesapeake Bay and Alaskan sea water samples. The samples were introduced into a graphite furnace and were analyzed by graphite furnace atomic absorption spectrometry. Analysis of concentrations below ng/mL for the trace elements mentioned was possible using this combination of sample preparation and instrumental analysis and no interelement interferences occurred. The combination of this sample preparation technique and the graphite furnace atomic absorption sensitivity enables extreme detection limits to be achieved for the elements mentioned, and gave measurements that were reliable and reproducible. The compatibility of the separation method when applied to spark source mass spectrometry was also demonstrated for thirteen transition elements, concentrated and separated from the sea water matrix.

The technique for the elimination and concentration of trace transition elements from the salt matrix has advantages in sample preparation for other analytical instrumental methods, in addition to the ones tested. Several of these instrumental methods could not be utilized without such a technique to remove the alkali and alkaline earth elements from sea water (neutron activation analysis and optical emission spectrometry) while others could reduce these interferences and be used more effectively with the separation and concentration of the sample prior to analysis (flame atomic absorption and x-ray fluorescence).

Ultra-purification techniques were employed in the preparation of the reagents used in this method; the analytical blank was found to be below detectable limits of graphite furnace atomic absorption spectrometry.

A technique was developed for redissolving neutral sea water samples after freezing or evaporation, utilizing the addition of CO_2 which is lost in freezing and evaporation of the sea salts.

FIGURES

Number		Page
1	The apparatus used for holding and delivering large quantities of sea water at a controlled rate to Chelex 100 resin. The apparatus (excluding column and clamp) was fabricated from Teflon FEP (*) or Teflon TFE (†), which has desirable anti-wetting and non-contaminating properties	15
2	The comparison of the removal of the alkali elements Na and K by ammonium acetate and ammonium nitrate from a Chelex 100 resin column which has previously chelated 100 mL of sea water	30
3	The comparison of the removal of Ca by ammounium acetate and ammonium nitrate from a Chelex 100 resin column which has previously chelated 100 mL of sea water	31
4	The comparison of the removal of Mg by ammonium acetate and ammonium nitrate from the Chelex 100 resin column which has previously chelated 100 mL of sea water	32
5	The elution of Ca, Mg, and Mn from a preconcentrated 50 mL sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent at pH 3.0, 4.5, and 5.0 and 2.5 M nitric acid stripping agent	34
6	The elution of Na, K, Ca, and Mg from a preconcentrated 100 mL sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent and 2.5 M nitric acid stripping agent	36
7	The elution of Na, K, Ca, and Mg from a preconcentrated 1 Liter sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent and 2.5 M nitric acid stripping agent	44

TABLES

Number		Page
1	The Gradient Elution of Na and K from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Nitrate Separating Agent and 2.5 M Nitric Acid Stripping Agent	23
2	The Gradient Elution of Ca and Mg from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Nitrate Separating Agent and 2.5 M Nitric Acid Stripping Agent	24
3	The Elution of Mn from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column Subjected to 1.0 M (pH 5.2) Ammonium Nitrate Separating Agent and 2.5 M Nitric Acid Stripping Agent	25
4	The Gradient Elution of Na and K from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	26
5	The Gradient Elution of Ca and Mg from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	27
6	The Elution of Mn from a 100 mL Sea Water Sample Preconcentrated on a Chelex 100 Resin Column Subjected to 1.0 M (pH 5.2) Ammonium Nitrate Separating Agent and 2.5 M Nitric Acid Stripping Agent	28
7	The Quantity of Na, K, Ca, and Mg Found in Various Effluent Fractions Substituting Water for a Separating Agent in the Elution from a Preconcentrated 100 mL Sample of Sea Water on a Chelex 100 Resin Column	37
8	The Amount of Na, K, Ca, and Mg Remaining in the Final 2.5 M Nitric Acid Volume After Elution of the Resin Column with 40 mL of Water, Ammonium Nitrate or Ammonium Acetate. The Column had Previously Preconcentrated a 100 mL Sea Water	38

TABLES (Continued)

Number		Page
9	The Elution of Na from a Preconcentrated 1 Liter Sample of Sea Water on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	40
10	The Elution of K from a Preconcentrated 1 Liter Sample of Sea Water on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	41
11	The Gradient Elution of Ca from a Preconcentrated 1 Liter Sample of Sea Water on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	42
12	The Gradient Elution of Mg from a Preconcentrated 1 Liter Sample of Sea Water on a Chelex 100 Resin Column Using 1.0 M (pH 5.2) Ammonium Acetate Separating Agent and 2.5 M Nitric Acid Stripping Agent	43
13	Summary of the Radio Tracer Study Results Showing the Percentage of Elution in Each of the Effluent Fractions for the 100 mL Samples	47
14	Summary of the Radio Tracer Study Results Showing the Percentage of Elution in Each of the Effluent Fractions for the 1 Liter Samples	48
15	The Concentration of Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn Found in Ten Identical 100 mL Sea Water Samples from the Chesapeake Bay as Determined by GFAAS After Chelex 100 Concentration and Separation	54
16	The Concentration of Na, K, Ca, and Mg Determined in the 2.5 M HNO_3 Effluent of the Chesapeake Bay Samples After Analysis of the Eight Transition Elements; Sample 3 was Spiked, Sample 8 was Unspiked and Sample 13 was a Blank	55
17	The Concentration of Cd, Mn, Ni, and Pb Found in Fifteen Different Sea Water Samples of 100 mL Each, Taken from Glacier Bay, Alaska as Determined by GFAAS After Chelex 100 Resin Column Concentration and Separation	56

ACKNOWLEDGMENT

The author is grateful to the U. S. Environmental Protection Agency for partial support of this work under the Interagency Energy/Environment Agreement EPA-IAG-D5-E684. This work is from a dissertation submitted and accepted by the graduate school, The American University, by H. M. Kingston, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

1. INTRODUCTION

One of the major emphasis areas of the measurements and instrumentation component of the Interagency Energy/Environment Program is the development of the capability to make baseline measurements in environmental systems where future energy related activities are expected to occur which may lead to changes in the state of the environment. The offshore area has been identified as one where the impact of energy related activities may occur and where extensive baseline measurements will be required. These measurements will consist of determination of levels of trace elements and organic constituents in the seawater, in the sediment, and in the biota. The Proceedings of the Workshop on Measurement Methods and Standard Reference Materials for Offshore Drilling-Petroleum, published as part of this Energy/Environment Series, recommends a number of high priority measurement method and Standard Reference Material needs related to the ocean waters, sediments, and biota. The current interest and expansion of offshore mining and oil exploration has the potential to alter the concentration of many of the trace transition metals found in seawater. The determination of the levels of these metals prior to any offshore energy related activities requires analysis techniques which are extremely sensitive. In addition, accurate analytical data on the current concentrations of the trace elements is essential for future monitoring of the metal concentration trends in sea and coastal waters. These measurements are extremely difficult since it is necessary to measure trace toxic element concentrations in the presence of considerably higher levels of alkali and alkaline earth elements.

In environmental samples, Na, K, Ca and Mg are usually present many orders of magnitude above the trace elements of interest; the most extreme example of such a matrix is sea water. Sodium, K, Ca and Mg are present in concentrations of 10.7, 0.399, 0.412 and 1.29 grams per liter, respectively. While the salinity of the water may change with location, depth or season, the ratio of these ions to one another is "conservative" and stays remarkably constant. However, the trace transition metals are present in microgram and submicrogram quantities and do not display the conservative relationship of the major elements (salts) of sea water (1). Appendix A contains a table of average concentrations of elements in sea water (1).

The major salts of saline waters are the normal ionic constituents of all natural waters, whether they are streams, lakes or the oceans into which they all eventually accumulate. The usual changes in their concentrations do not disrupt aquatic life forms since their major contribution is a salt gradient. This is not true of the minor cations which may cause undesirable effects on many biological systems. The transition metals are of great interest biologically because small shifts in their concentration can cause significant biological effects; they may also have adverse effects on humans who consume the products of the sea, as many transition metals bioaccumulate to much higher than ambient levels (2).

This paper describes a technique which was developed for the elimination of the alkali and alkaline earth elements from the trace transition elements in seawater samples by the use of a Chelex 100 Resin Seawater sample chelation. This technique was found to have excellent recoveries of most trace toxic elements and was tested on a variety of seawater samples, with the analysis being performed by a variety of common techniques. In addition, in order to evaluate methods for preparing and storing a seawater Standard Reference Material, a technique was developed for redissolving neutral seawater samples after freezing or evaporation, utilizing the addition of CO_2 which is lost in freezing and evaporation of the sea salt.

2. SUMMARY AND CONCLUSIONS

The overall purpose of this study was to develop a technique by which complete separation of the alkali and alkaline earth elements (Na, K, Ca and Mg) from the trace transition metals (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) could be accomplished in a sea water matrix.

In fulfilling this purpose, five specific objectives were considered:

-Develop a separating agent technique capable of stripping the Na, K, Ca and Mg from a column of Chelex 100, which has chelated trace transition metals from sea water, without removing the transition metals of interest.

-Determine the parameters (pH, resin volume, flow rate) which will allow the maximum efficiency for the preconcentration and separation.

-Evaluate the efficiency of the preconcentration and separation for the transition metals of interest.

-Establish conditions which will reduce the analytical blank of these preconcentration and separation procedures to an acceptable value.

-Preconcentrate and separate actual sea water samples and determine the actual usefulness on instrumentation normally suffering from interelement effects rendering them incapable of this application, specifically, graphite furnace atomic absorption spectrometry (GFAAS) and spark source mass spectrometry (SSMS).

The attainment of these objectives provides a procedure enabling the elimination of alkali and alkaline earth elements from trace transition elements. This procedure not only provides the means for sea water analysis by instrumental techniques previously inhibited by the sample matrix, but also establishes a basis for further research by applying this combination of preconcentration and separation to other samples presently prohibited from instrumental analysis by similar matrix interferents.

A 1.0 M ammonium acetate solution has proven capable of stripping Na, K, Ca, and Mg selectively from a column of Chelex 100 resin which has chelated up to a 1 liter sea water sample. During the removal of the alkali and alkaline earth elements the trace transition elements chelated by the Chelex 100 resin are unaffected by the ammonium acetate separating agent.

The pH range of 5.0-5.5 appears to be the most suitable for both the concentration of the trace transition elements onto the Chelex 100 resin column and for the removal of the alkali and alkaline earth elements from the resin using ammonium acetate. The pH in this range affords ample separation between the elution of the salt matrix and the transition elements attaining a complete separation between these two elemental classes.

The concentration and recovery is quantitative for Cd, Cu, Mn, Ni, and Zn, being >99 percent efficient. The Mn ion can only be collected quantitatively from volumes less than 500 mL using the present resin volume. The recovery of Co and Pb is >99 percent and >98 percent, respectively, at the 100 mL sample volume and >97 percent at the 1 liter level. These recoveries would appear to be sufficiently quantitative for most instrumental techniques. Many instrumental techniques have uncertainties in measurement of 5 percent. Both the SSMS and GFAAS have uncertainties greater than the small losses detected in the concentration step of the present procedure. The SSMS would, however, not be affected by any losses of the magnitude involved in the ions investigated here, including Fe. The SSMS technique would not have the sensitivity to measure all the elements tested by GFAAS at the levels in the samples utilized in this study. For many of the elements in these sea water samples the GFAAS operated near its detection limits and would introduce more uncertainty into the measurement than the recoveries of the analytes during the concentration technique. Iron was not reproducibly retained by the Chelex 100 resin and varied in its recovered efficiency. Instrumental procedures which depend on total recovery for quantization should not rely on the recovery of Fe, being more reproducible than about 2 percent due to air oxidation alteration of the Fe^{+2}/Fe^{+3} ratio.

The ammonium acetate can be eliminated from the column with only several resin volumes of water excluding it from any addition to the final sample volume. The ammonium ion can be eliminated from the final volume, thus rendering the separating agent innocuous to all instrumental applications. The elimination of the ammonium ion from the final volume, when used in conjunction with GFAAS, proved to be unnecessary as it produced no interference as ammonium nitrate in the final volume.

The analytical blank, as determined by GFAAS for the entire procedure, was low enough to be undetectable for every element tested, with the exception of Fe (which was discussed). The blanks are well within acceptable limits to avoid any interference with even pristine Alaskan sea water samples. Perhaps, as the instrumental techniques are improved and lower detection limits are achieved, other steps may be necessary to decrease these levels. At the present time, however, the reagent purification procedures, the laboratory environment, and procedural precautions used are adequate to eliminate the analytical blank as a source of error in the analysis.

The use of this separation method has enabled the GFAAS and SSMS instrumental techniques to become compatible with the Chelex 100 preconcentration method for high salinity samples. The final samples produced no interelement interferences when applied to GFAAS and enabled the maximum instrumental sensitivities to be used. The method was also compatible for multielemental analysis using SSMS. These successful instrumental trials can be extrapolated to other instruments which require similar matrix elimination and also to those which, to the present time, have made use of the Chelex 100 transition metal concentration with moderate interferences. The method in its present form appears to be ready for practical use, and should enable analysis such as those presented here to become of only routine complexity.

The significance of the development of this technique has two important aspects. First, the separation and concentration technique enables the use of instruments previously unable to measure the trace transition metals in saline waters. Several of these instruments such as GFAAS possess extremely low detection capabilities for the elements. Others, such as neutron activation analysis, possess multielemental character and superior sensitivity. Second, using instrumental techniques which possess these low sensitivities, studies of the extremely low levels of the trace transition elements in saline waters can be done with accuracy. Measurement of the trace elements in pristine saline waters can be achieved and true baseline data collected for environmental assessment of man's influence on the oceans.

3. LITERATURE REVIEW

The literature of marine water analysis reflects the considerable difficulty in establishing an accurate and precise method of analysis for trace metals in saline matrices. The complex and highly concentrated saline matrix defies a simplified approach for trace metal analysis.

There are no analytical techniques currently available for the direct determination of elements in sea water at concentrations below 5 $\mu g\ L^{-1}$ (3). Usually it is essential to concentrate the trace elements from a large volume and separate the transition elements from the alkali and alkaline earth elements. In such sample preparations the efficiency of concentration, completeness of separation, and total analytical blank become critical to the final instrumental method (3-6).

Current preconcentration techniques include co-precipitation (7), chelation and extraction (8,9), and chelation ion-exchange resin (4,10). These techniques used for the preconcentration of trace elements from sea water have been reviewed by Riley and Skirrow (3).

The preconcentration technique which has come into prominence during the past seven years has been the chelating ion-exchange resin technique, utilizing Chelex 100 resin (refined by Bio·Rad Laboratories from Dowex A-1 resin). The use of Chelex 100 to concentrate trace transition elements from sea water has been found to be highly efficient, while exhibiting low analytical blanks (3,4).

Lai, Callahan and co-workers first demonstrated the practical use of Chelex 100 resin for the concentration of Ag, Co, Fe and Zn from quantities of saline water exceeding 50 liters (11,12). A column of Chelex 100 (50-100 wet mesh) was contained in a 2.2 cm X 7.6 cm bed and the flow rate was 33 mL/minute, or approximately 2 liters per hour. The retention on the Chelex 100 resin was 54 percent for ^{110m}Ag, 92-95 percent for ^{59}Fe, 95-99 percent for ^{60}Co and 100 percent for ^{65}Zn. The elution of the ions from the elution of the ions from the column was quantitative, with high concentrations of HCl (3-5 M). The acid elution contained quantitatively all of the trace metals chelated by the column, within their experimental error (except for Ag). A quantity of the major salts Na, K, Ca and Mg were also eluted in the acid effluent. The concentration of the major ions in the final fraction was several orders of magnitude greater than that of the heavy metals. There was no attempt to separate the ions collected and all ions were eluted simultaneously.

Quantitatively, 95-99 percent of the Co was found to be retained from sea water by the Chelex 100. This was attributed to the existence of both Co^{+2} and Co^{+3} in sea water; the Co^{+2} was chelated quantitatively, while the Co^{+3} was not quantitatively retained. Cobalt exists in sea water principally in the divalent state (about 96%). A similar incomplete chelation was observed for Iron, for the same reason. Zinc was quantitatively retained, being present as Zn^{+2} in natural sea water. The optimum results were obtained at pH 5.0.

In 1968 Riley and Taylor wrote a series of three closely related papers investigating the use of Chelex 100 resin for the preconcentration of trace transition metals in sea water and the utilization of this technique for sample preparation in flame atomic absorption and spectrophotometry (13). The resin was used in the hydrogen form and 50-100 mesh size. The column resin was packed in a 1.2 cm glass tube to a height of 6 cm. The flow rate was not allowed to exceed 5 mL/min. A 1 liter sample of 0.5 μ membrane-filtered sea water was spiked with the radiochemical tracers or natural elemental tracers, adjusted in pH from 5.0 to 9.0, and concentrated on the resin. Evaluation of the quantitation was made either radiochemically or photometrically. The investigation reported 99-100 percent retention and subsequent elution of Bi, Cd, Co, Cu, In, Mn, Mo, Ni, Pb, Re, Sc, Th, W, V, Y and Zn. The eluting agent was 30 mL 2 N HNO_3 for all elements except Mo, Re, W and V, which were chelated as anion oxides and were removed with 20 mL 4 N NH_4OH. Prior to elution, the column was washed with 20 mL of distilled water. The preconcentration eliminated most of the alkali and alkaline earth elements, leaving 80 and 1.8 mg Na, 1 and 0.3 mg K, 2.3 and 0.2 mg Ca, and 0.3 and 0.02 mg Mg remaining on the column, which were eluted with 2 N HNO_3 and 4 N NH_4OH, respectively. There was no attempt to separate the alkali and alkaline earth elements that remained in the trace metal effluent.

In two later works in 1968, Riley and Taylor recommended the ammonium form of Chelex 100 (14,15) rather than the hydrogen form used in their original paper. They found that low pH retention of the resin in the lower portion of the column caused losses in the first 100 mL of sea water until the pH of the resin increased to at least pH 5.0.

Since the developmental work of Riley and Taylor using Chelex 100 for the concentration of trace metals from sea water, this technique has been used by many researchers without significant alteration. The preconcentration technique has been utilized for sample preparation to increase the concentration of trace elements to detectable limits for flame atomic absorption spectrometry, spectrophotometry, pulse polarography, anodic stripping voltammetry, and x-ray fluorescence.

The flame atomic absorption analysis technique has been utilized for Cd, Co, Fe, Mn, Ni, Pb and Zn, collected from marine water using Chelex 100 (4,13, 15-19).

Spectrophotometry has utilized this concentration technique for Mo, V (14), Cd, Cu, Fe, Mn, Ni and Zn (14,20).

Pulse polarography was employed to determine Cd, Co, Cu, Ni and Zn in marine samples using Chelex 100 to preconcentrate the trace metals (21).

Anodic stripping voltammetry has utilized Chelex 100 for the concentration of Cd, Cu, Pb and Zn before analysis (22,23).

X-ray fluorescence has likewise utilized the concentration of Cu, Mn, Ni and Zn from a 3 liter sample prior to irradiation to increase the concentration of these metals to within detectable limits (24).

Neutron activation analysis has used Chelex 100 to concentrate the transition metals Ba, Cd, Ce, Co, Cr, Cu, Fe, La, Sc, U, V, and Zn (25).

The possibility of other naturally occurring chelators competing with the Chelex 100 resin has been investigated to evaluate the effect they could have if they occurred in high concentration in a natural water sample. Glycine, histidine and photoplankton were added to marine water in concentrations as high as 10^{-5} M. No competition with Chelex 100 was found. Only EDTA at concentrations of 10^{-6} M or higher showed any effective competition for ^{64}Cu (4). Other organic acids were also introduced in 2 to 250 mg/liter concentrations (citric acid, malic acid, palmitic acid, p-hydroxybenzoic acid, glycine, humic acid, aspartic acid, leucine, l-cystine, NTA and EDTA). Only the synthetic chelator EDTA exhibited any competing chelation for Cu or Zn (23). The natural chelators do not compete favorably with Chelex 100, thus explaining in part the excellent recoveries reported for transition metals concentrated from natural water systems.

The ability of Chelex 100 to chelate the transition metals from a solution that contains Na and K 10^8, and Ca and Mg 10^6 times that of the trace elements stems from a large range in selectivity coefficients. Using Zn^{+2} as a standard point and giving it a value of 1.000 in selectivity, Na^+, Ca^{+2} and Mg^{+2} would chelate with a strength of 10^{-7}, 1.3×10^{-2} and 9×10^{-3}, respectively. This is in comparison with 1.3×10^{2} for Cu^{+2}, 4.4 for Ni^{+2}, 3.9 for Pb^{+2}, 4×10^{-1} for Cd and 2.4×10^{-2} for Mn (26). (Appendix B contains a more complete selectivity series.)

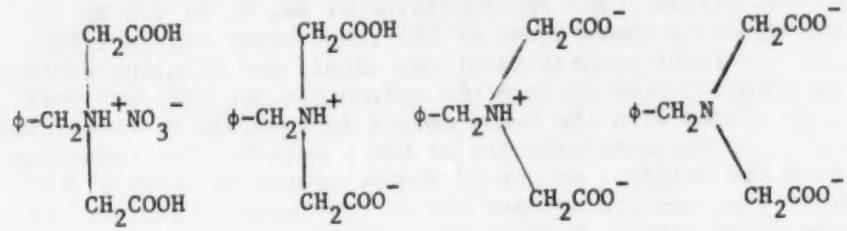
The order of selectivity for cations in nitrate or chloride media is:

$$Cu^{+2} >> Pb^{+2} > Fe^{+3} > Al^{+3} > Cr^{+3} > Ni^{+2} > Zn^{+2} > Ag^{+} > Co^{+2} > Cd^{+2} > Fe^{+2} > Mn^{+2} > Ba^{+2} > Ca^{+2} >>> Na^{+}$$ (26).

The selectivity series for cations in an acetate buffer system at pH 5.0 is:

$$Pd^{+2} > Cu^{+2} >> Fe^{+2} > Ni^{+2} > Pb^{+2} > Mn^{+2} >> Ca^{+2} = Mg^{+2} >>> Na^{+}$$ (26).

The selectivity of the iminodiacetate resin has been shown to be highly pH sensitive. The divalent metal ions experience both ion exchange and chelation. Both of these effects are pH dependent because they depend upon the number of available ionic sites inside the resin particle and on the shape of the functional group, which also changes with pH. Titration of Chelex 100 produces the following zwitterionic forms as a function of pH (27).



At lower pH, the main effect is on ion exchange with each divalent ion neutralizing the charge on two ionic sites, each of which is furnished by a different diacetate group. Ion exchange increases with pH as more ionic sites become available by migration of hydrogen ions out of the resin until a pH of approximately 4 is reached. At this point chelation can start. The half neutralization point (50% dissociated) inside the resin has been shown to occur at a pH of approximately 5.6 $\pm$ 0.2 (28). It has been found, however, that chelation becomes effective at a lower pH as ionic strength increases (29). When the distribution coefficient was plotted vs. the pH, the transition metals exhibited curves similar to those of titration curves having the familiar "S" shape. They begin to rise in distribution coefficient value at a pH of approximately 3.0 and have leveled off at a maximum value by pH 5.0. The distribution coefficients appear to remain at a maximum value with increasing pH, with the exception of Co and Cu, which begin a decline of the distribution coefficient at approximately pH 6.0 (28,30). The curve for the alkaline earth elements Ca and Mg was found to have a plateau from pH 4 to 5.8 (31). When studied in a high ionic strength medium, this non-increasing region of the plot of the distribution coefficient vs. pH was found to decrease to a minimum between pH 4.0 and 5.8 and then began rising rapidly to a maximum distribution coefficient value at pH 5.8 (29). When compared, these several studies suggest that a defined working range of pH 5 to 6 is available to take advantage of the maximum distribution coefficients of the transition metals and also to utilize the minimum value for Ca and Mg in higher ionic strength solutions.

It has been concluded by other researchers that the differences in stabilities for divalent ions are not significant enough to allow a complete separation of one element from another (3). In a study of Chelex 100, Van Willigen et al. (32) concluded that because of insufficient stability differences, swelling with pH and insufficient equilibration time, efficient separation of metals was not feasible. In an attempted separation of Ca from Cu, complete dseparation was not accomplished.

At present the trace transition metals of saline waters are being concentrated and eluted with acid without any further attempt to separate the large quantities of alkali and alkaline earth elements before stripping them collectively from the Chelex 100 column (3).

The origins of these alkali and alkakine earth elements are the residual sites on the resin not taken up by the trace transition metals; these residual sites concentrate the salt matrix. The concentration of Na, K, Ca and Mg remains many orders of magnitude above those of the trace heavy metals after preconcentration (3,13). Further separation of the alkali and alkaline earth elements beyond the salt that passes through the column has not been achieved. Thus, the amount of salt eluted with the heavy metals is governed by the minimum amount of resin required for the concentration of heavy metals. The reduction of Na, K, Ca and Mg from the original amount in the sea water is accomplished by using much less resin than can accommodate all of the ionic content of the saline water. The transition metals, being held several orders of magnitude more strongly by the Chelex 100 than the major salts, are quantitatively chelated while the bulk of the salts pass through the column. Thus, the major salts normally eluted with the trace metals are those occupying the remainder of the resin capacity above the requirements of the heavy metals (3,13,21).

The use of this trace metal concentrate with a high salt content is limited to instrumental techniques which can tolerate these large quantities of Na, K, Ca and Mg eluted with the trace metals. These techniques which utilize the Chelex 100 resin preconcentration have been previously described. They are not, however, the most sensitive, nor are they multielemental techniques, with the exception of neutron activation analysis and x-ray fluorescence. (X-ray fluorescence has not been well quantified using conventional techniques.)

The method of x-ray fluorescence quantification described by Campbell et al. utilizing the SA-2 resin loaded filter paper disks, cannot be implemented while even small quantities of Na, K, Ca or Mg are present in the sample matrix. The alkali or alkaline earth elements, in combination with the trace metals, destroy the analytical application of this technique through competition for sites in the resin loaded paper used for quantification (33-35).

Spark source mass spectrometry, although independent of recovery for analytical analysis, cannot tolerate the alkali or alkaline earth elements in concentrations much above those of the trace elements being analyzed, making necessary a separation selectively removing the higher concentrations of these salts while retaining the trace elements of interest (36).

Optical emission spectrometry, using inductively coupled plasma or electrode plasma (D.C. arc), exhibits interelement interferences when Na, K, Ca or Mg are introduced at levels much higher than those of the trace elements under study (37-39).

In neutron activation analysis the problem of interference from the salt matrix is not so severe since Na is the only major interferent.

Sodium, upon radiation, produces ^{24}Na, necessitating radiation shielding and remote handling, as well as interfering with short-lived isotope counting.

Two techniques using Chelex 100 have been employed to overcome this problem. Forberg and Lundgren (40), placed a sample on Chelex 100 after irradiation, replaced ^{24}Na with natural Na, and counted the column. Most recently, Lee, et al. (25) used a mixture of Chelex 100 and ground glass to preconcentrate the trace metals from 1 to 4 liters of sea water, which had been filtered with a 0.5 μ filter and stored acidified to pH 1 with nitric acid. After adjusting the pH to 8.0 and preconcentrating the sample, they eluted the Na, Br, K and Cl using 100 mL of 0.01 M nitric acid and eluted the trace metals with 50 mL of 4 M nitric acid. They were able to recover ^{115m}Cd, ^{64}Cu, ^{56}Mn and ^{64}Zn from 99.7 to 100.2 percent. This technique did not remove Ca or Mg prior to the final elution, but due to their non-interfering radiations, these large quantities of alkaline earth metals did not interfere with the counting, utilizing both the NaI(Tl) and Ge(Li) detection equipment.

One of the most sensitive methods of elemental analysis is graphite furnace atomic absorption spectrometry (GFAAS). It is possible to detect 10^{-12} to 10^{-9} g of many of the trace elements in marine water. It would appear from the detection limits alone that this technique could be used directly on marine water samples for some of the trace metals. However, the high salt content of sea water (35g/kg) makes it difficult to effectively volatilize the matrix without loss of the analyte. The major component of sea water is sodium chloride, which has a relatively high volatilization temperature. Also, the trace metals in sea water are present as chlorides, which have a lower volatilization temperature. Therefore, it is difficult to volatilize the sodium chloride during the ashing step without losses of the analyte. Calcium and magnesium chlorides are present in large quantities and a temperature greater than 2000 °C is required to volatilize these elements. Thus, even if the sodium chloride is removed during the ashing step using matrix modification (41), which requires interference corrections, calcium and magnesium chloride remain to interfere with the analyte during atomization (42,43). When the Chelex 100 column preconcentration of the trace elements is employed, the Na, K, Ca and Mg are present in the final acid solution with the trace metals at levels almost equal to the original sea water matrix (13). Even if the alkalis are removed, the calcium and magnesium cause depression of the signal from two to ten times, depending on the analyte (43). This effectively decreases the normal detection advantage of the GFAAS method. Thus, the interelement effects from the salt matrix in both the original marine water or the Chelex 100 concentrate render the GFAAS technique incompatible for these samples.

In summary, the application of the Chelex 100 trace metal concentrate from marine waters has been instrumentally limited. The residual salt content eluted with the trace metals approximates the concentrations in the original sea water. These high concentrations of alkali and alkaline earth elements have denied the use of instrumental techniques which suffer interelement effects from this salt matrix. The present use of Chelex 100 has achieved an increased concentration of trace metals for instrumental techniques able to utilize samples with a high salt content. If the preconcentration were extended to a complete separation, eliminating the alkali and alkaline earth elements, the matrix free samples would permit the utilization of a variety of instrumental techniques presently excluded from trace metal analysis of saline samples. Many of these instrumental techniques have characteristics that would enable the analysis of trace metals with greater sensitivity and multielemental efficiency.

4. EXPERIMENTAL PROCEDURES

MATERIALS

Chemical Reagents

Water, nitric and glacial acetic acids were prepared using sub-boiling distillation at the National Bureau of Standards (NBS), as utilized in ultra-purification procedures at NBS (44). All reagents used in the separation process were prepared and stored in clean FEP Teflon, unless otherwise stated. Water and acid, unless otherwise specified, are these high purity reagents. (Appendix C lists typical analyses for HNO_3 and H_2O prepared using this technique.)

Ammonium hydroxide was prepared by bubbling filtered ammonia gas through sub-boiling purified water until room temperature saturation was achieved.

A 1.0 M solution of ammonium acetate was prepared by mixing 60 g of purified glacial acetic acid and 67 g of saturated ammonium hydroxide and diluting to 1 liter in a polypropylene volumetric flask. The acidity was adjusted to pH 5.0-5.3 by dropwise addition of HNO_3 and/or NH_4OH.

All reagent and sample preparations were done in a class 100 clean air laboratory (45).

Chelex 100 chelating resin, 200-400 mesh size, was purchased from Bio·Rad Laboratories.

The elemental tracers ^{59}Fe, ^{54}Mn, and ^{65}Zn in 0.5 N HCl were purified reagents obtained from the Chemical and Radioisotope Division of ICN. The ^{60}Co, and the short lived isotopes, ^{64}Cu and ^{65}Ni, were made by the Neutron Activation Analysis Section at NBS from five-9's pure metals and dissolved in nitric acid. The ^{109}Cd and ^{210}Pb were obtained by the Activation Analysis Section from other sources and counted for purity before use.

All standard stock solutions for atomic absorption spectrometry (AAS) were prepared from high purity metals or salts in sub-boiling distilled NBS acids, as described by Dean and Rains (46). Working solutions were prepared as needed.

The buffer solutions used for pH standardization were 0.050 m potassium hydrogen phthalate (pH = 4.008 at 25 °C) and 0.025 m disodium hydrogen phosphate and 0.025 m potassium dihydrogen phosphate (pH = 6.865 at 25 °C). These reagents were prepared from ACS analytical grade reagents.

Chesapeake Bay Sea Water

The sea water used for method development was obtained during high tide at the Virginia Institute of Marine Science (VIMS), at Gloucester Point on the Cheaspeake Bay. The source was a submersible pump and plastic tubing permanently submerged approximately 100 meters off shore from the Institute. This sampling system has been in continuous operation for three years; the continuous sea water supply is normally used to support live laboratory experiments at VIMS. The sea water was pumped directly into a conventional polyethylene drum which had been cleaned first with hydrochloric and then with nitric acid and then with purified water prior to use (47). After filtration filtration
through a 0.45 μ millipore filter using an all polypropylene filter apparatus, the sea water was collected in a clean polyethylene carboy and acidified with Ultrex HNO_3 to prevent bacterial growth and to stabilize the trace element concentrations (48,49).

Glacier Bay Alaskan Sea Water

A collection of pristine sea water samples was obtained by NBS personnel for analysis. The ship "Surveyor" was used to transport men and equipment from Juneau to the selected sample site, Glacier Bay, Alaska. Samples were collected using a Teflon sampler and sampling technique developed at NBS (50). One liter Teflon (FEP) bottles were prepared prior to sampling by cleaning in hot (1:1) HCl for twenty-four hours and then in hot (1:1) HNO_3 for a second twenty-four hour period, after which they were thoroughly rinsed in sub-boiling distilled water (44). A 44 g volume of NBS high purity HNO_3 was added to the sea water sample to bring a 1 liter sample to 0.5 N in HNO_3 (48). The sea water sample was then placed directly in the bottles at the sample site to avoid contamination and the bottles were individually sealed in polyethylene bags. The samples were transferred to a large freezer, maintained at -40 °C, and were kept there until needed for analysis. These samples were designated "NBS, 500 through 503, a and b".

A second group of samples also taken at Glacier Bay, Alaska were collected by other researchers under varying conditions and transported to NBS. These frozen samples arrived at NBS in varying states of acidity and filtration. These samples were designated "Alaska, 1 through 5, a and b". The samples were in polyethylene containers without polyethylene bags.

APPARATUS

Column Separation Apparatus

An Isolab QS-Q polypropylene column with porous polyethylene resin support was used for 100 mL and 1 liter sample volumes. Although the same column was used for both sample volumes, the amount of resin and reservoir

systems was entirely different. For the 100 mL sample, the QS-S 25 mL conventional polyethylene extension funnel was attached to the column to act as a reservoir for the sample.

For a 1 liter sample the reservoir was a 1 liter Teflon (FEP) bottle inverted and modified with a machined Teflon (TFE) closure insert containing a microbore venting tube and outlet tube. The outlet was connected to a valve (TFE) by a 1.59 mm i.d. Teflon (FEP) tubing and (TFE) connector linked to the reservoir with a specially machined mount (TFE) which sealed the column into the closed system. The mount contained a vent (sealed with a nylon screw, allowing the removal of air from the system) as well as an inlet and was tightly clamped to the column using the lip on the column at point B (see Figure 1). The clamp (a modified glass joint clamp) and mount provided a seal hich allowed the reservoir to be raised above the column to obtain enough pressure to control the flow rate using the pressure of the raised reservoir and the valve (Figure 1).

Atomic Absorption Spectrometry Apparatus

The initial experiments to determine the parameters necessary to separate the alkali and alkaline earth elements (Na, K, Ca and Mg) from the transition metals were analyzed utilizing flame atomic absorption. Manganese, being the most weakly held of the transition metals, was used to trace their location. Both atomic absorption and atomic emission were used in these determinations. The Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer, equipped with Automatic Burner Control System, was used. Use was made of a Perkin-Elmer 3-slot Boling Burner Head with premix chamber and pneumatic nebulizer for burner requirements. The expanded scale in the concentration mode, utilizing either 10 or 100, averaging reading selection was used. The gas mixture was either nitrous oxide-acetylene or air-aceytlene. All instrumental parameters were adjusted to those suggested in the Perkin-Elmer instrument manual (51).

A Jerrell Ash Czerny-Turner Scanning Spectrometer, with wavelength modulation for background correction (0.75 meter) was also used. It was equipped with a Perkin-Elmer single slot burner having a pneumatic nebulizer and premix burner chamber. A Perkin-Elmer concentration readout DC R2B was used for quantitative readout. A Princeton Applied Research lock-in amplifier with Fluke high voltage power supply was used for the photomultiplier tube. An air-acetylene or nitrous oxoide-acetylene flame was used and the wavelength and burner parameters were those supplied by the Perkin-Elmer atomic absorption manual (51).

The Hewlett-Packard Model 9830B Desk Top Computer was used to perform a polynomial regression on the calibration data and to calculate the concentrations of Na, K, Ca, Mg and Mn in the test solutions. The Hewlett-Packard Calculator Plotter Model 9830A was used to plot the data, and the concentration values were printed on a Hewlett-Packard 936613 Printer. The program was Polynomial Regression File 3, as listed in the technical manual (52).

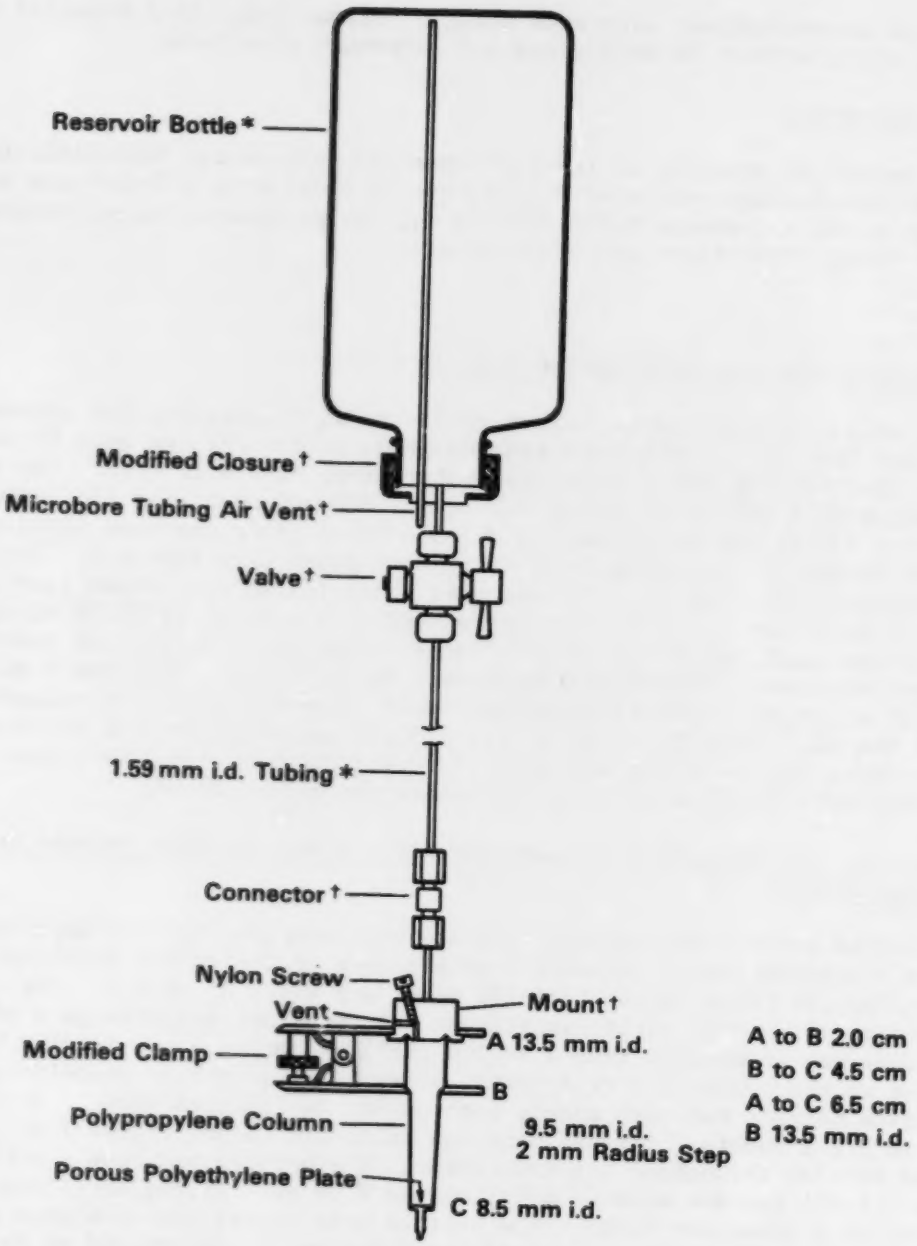


Figure 1. The apparatus used for holding and delivering large quantities of sea water at a controlled rate to Chelex 100 resin. The apparatus (excluding column and clamp) was fabricated from Teflon FEP (*) or Teflon TFE (†) which has desirable anti-wetting and non-contaminating properties.

pH Apparatus

The pH determinations were done using a Beckman Model SS-2 Expanded Scale pH Meter, equipped with Beckman glass and reference electrodes.

Counting Apparatus

The gamma ray counting of the radiochemical tracers was done utilizing a sodium iodide-thallium activated 7.5 cm crystal coupled to a Fluke high voltage power supply and a Canberra Model 810 and 835 Energy Discriminator equipped with Ridl Model 27101 Timer and 27104 Counter.

PROCEDURE

Column Preparation and Purification Procedure

The column preparation procedure consisted of precleaning the columns in 1:4 HCl and then in 1:4 HNO_3 (ACS reagent grade acids) for one week in each bath and then rinsing the columns with water after each acid wash. The column was loaded with a slurry of Chelex 100 resin, 200-400 mesh size (in sodium form). For 100 mL sea water samples 3.2-3.4 mL of resin was used which covered the lower barrel of the column from point B to point C in Figure 1. For 1 liter samples 5.8-5.9 mL of resin was used which filled the column from point A to point C in Figure 1. The resin was washed with a total of 15-20 mL of 2.5 M HNO_3 (for the small and large resin volumes, respectively), in 5 mL portions to elute any trace metal contamination present in the resin. Then two 5 mL volumes of water were used to rinse the resin of excess acid. To transform the resin to the NH_4^+ form, 10-15 mL of 2.0 M NH_4OH was added in 5 mL volumes. After checking the pH of the effluent to assure the basicity, the column was then rinsed with 10-15 mL of water to remove the excess NH_4OH.

Procedure for the Comparison of Ammonium Nitrate and Ammonium Acetate as Separating Agents

Ammonium acetate and ammonium nitrate were compared for the separation of alkali and alkaline earth elements from a column of Chelex 100 which had preconcentrated the trace metals from 100 mL of sea water at pH 5.0. The reagents were prepared from high purity sources as described and adjusted to a pH of 5.2. The reagent was preweighed into a Teflon (FEP) beaker on a top loading balance and added to the column 5 g at a time. After 50 mL of either ammonium acetate or ammonium nitrate had been added, each column was treated with 15 mL of 2.5 M HNO_3 in 5 g aliquots. The effluent was collected in preweighed 7 mL polyethylene bottles throughout the experiment. The bottles had been cleaned one week in 1:4 HCl and one week in HNO_3, rinsed with sub-boiling distilled water and dried on a clean air bench. The bottles were capped and reweighed on a top loading balance. The effluent was analyzed for Na, K, Ca, Mg and Mn by atomic emission and atomic absorption spectrometry. Instrumental parameters were followed from the Perkin-Elmer atomic absorption instrumental manual (51).

Evaluation of the Influence of pH on the Separation

Six columns of Chelex 100 resin were prepared in the ammonium form. A 300 mL sea water sample was spiked with 10 mL of 10.079 µg/mL Mn. The sample was adjusted to pH 5.0 and divided into six identical 50 mL samples. Each sea water sample was passed through a separate Chelex 100 resin column and the effluent collected in a 60 mL polyethylene bottle. Five separate 70 mL volumes of 1.0 M ammonium acetate were adjusted, using nitric acid, to pH 3.0, 3.5, 4.0, 4.5, and 5.0. The different ammonium acetate fractions were added to the column in 6.0 mL volumes and one was treated with neutral water as a control. The effluent was collected in 6.0 mL portions in 7 mL precleaned polyethylene bottles. After 54 mL of the ammonium acetate had passed through the column, 6 mL of 2.5 M HNO_3 was added to each column and this effluent collected as before. The individual fractions from each column were analyzed for Na, K, Ca, Mg and Mn by atomic absorption spectrophotometry or emission spectroscopy using the Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer (AAS).

Column Preconcentration and Separation Procedure

After changes in the procedure during exploratory experiments, the procedure was standardized for further quantitative experimental investigation. For the 100 mL sea water sample 101.3 ± 0.2 g was weighed directly into a clean 250 mL Teflon (FEP) beaker. If a spike (natural or radiochemical) was to be added, it was added and then the pH adjusted to pH 5.0-5.5 with the dropwise addition of NH_4OH or HNO_3. Then 0.5 mL of purified 8 M ammonium acetate was added to aid in buffering the system in this range. Any necessary agitation of the solution was done with a Teflon stirring rod. A small amount of the sea water was added to the reservoir and column to allow the resin to undergo its natural shrinkage as it changed ionic form and pH. This shrinkage results in a resin volume of approximately one half of its original volume. After the completion of this transformation was observed (two to three minutes) the remaining sea water was added to the reservoir as needed to keep it filled. The flow rate was approximately 0.8 mL/min for the duration of the separation. To selectively elute Na, K, Ca, and Mg, and replace them with NH_4^+, 40 mL of 1.0 M ammonium acetate was added to the column in 10 mL volumes. At the completion of the ammonium acetate addition, a 5 mL volume of water was added to remove any residual ammonium acetate. The elution of the transition metals was then accomplished with 7 mL of 2.5 M HNO_3 collected into clean preweighed 10 mL conventional polyethylene bottles. The bottles were capped with clean polyethylene lined caps and reweighed to accurately determine the weight of effluent.

For the 1 liter samples the procedure was essentially the same as the 100 mL samples, with minor alterations due to the apparatus requirement. The sample, 1,013 g ± 0.5 g, was weighed into a 1 liter Teflon (FEP) bottle and the pH adjusted in the same manner as previously described. The bottle became the reservoir and was fitted with a modified closure (see Figure 1). The bottle was inverted and the air purged from the system by means of the vent on the column mount. The flow rate was adjusted using the valve and the height of the reservoir and was kept to less than 0.2 mL/min until the shrinkage of the resin was complete. Then the flow rate was increased to 1.0 mL/min and left overnight to flow through the column (approximately sixteen hours required).

After passing the sample through the column, the valve and tubing were removed at the connector above the column mount and replaced with a smaller reservoir containing 70 mL of 1.0 M ammonium acetate. The flow rate was adjusted to 0.5 mL/min and allowed to flow until the reagent was exhausted. The column was then removed from the mount and the resin washed with 10 mL of water. The transition metals were eluted with two 5 mL portions of 2.5 M HNO_3 into preweighed polyethylene bottles as previously described.

Radiochemical Procedure

Radiochemical tracers were used to gain specific information about the behavior of each transition metal ion in the preconcentration and separation with the Chelex 100 resin. The tracers were added to the sea water one radioisotope per sample prior to the pH adjustment, using disposable polypropylene micro-pipettes. The column procedure was identical in all respects to the preparation of the analytical samples previously described. However, all effluent from the column was collected, including the sea water. The sea water, ammonium acetate buffer, acid effluent, and column resin were collected in 250 mL bottles in the 1 liter experiments, and the 100 mL samples were collected in 125 mL bottles. The level of fluid in each bottle was adjusted to that of the largest member of that elemental series by the addition of distilled water on a top-loading balance, allowing normalization of the samples for counting purposes. All samples contained the same amount of fluid in identical containers. The bottles were counted for ten minute periods for gamma radiation only.

This technique was checked for total recovery using ^{65}Zn. The tracer was added to 250 mL of the sea water sample and counted prior to manipulation. This volume was then added to 750 mL of sea water and treated as a 1 liter ^{65}Zn spiked sea water sample. The final acid volume was again adjusted to 250 mL and counted at the end of the separation as previously described. The counting statistics for each element were optimized by energy discrimination. The statistical error was obtained using the following equation (53,54,55):

$$Q_c = 100 \times \frac{K}{N_s - N_b} \sqrt{N_s + N_b}$$

where

Q_c = Percent experimental error corrected for background

K = Number of standard deviations

N = cpm = counts per minute or period unit time

s = sample including background

b = background

Evaluation and Application of the Separation Technique by Spark Source Mass Spectrometry

Eight 101.3 g sea water samples were weighed into 150 mL Teflon (FEP) beakers. Samples #1 and #2 were not spiked. Samples #3 and #4 were spiked with 10^{-7} g of the stable isotopes ^{26}Mg, ^{41}K, ^{44}Ca, ^{47}Ti, ^{53}Cr, ^{54}Fe, ^{62}Ni,

^{65}Cu, ^{67}Zn, ^{71}Ga, ^{82}Se, ^{86}Sr, ^{91}Zr, ^{97}Mo, ^{109}Ag, ^{110}Pd, ^{111}Cd, ^{113}In, ^{117}Sn, ^{125}Te, ^{137}Ba, ^{142}Ce, ^{145}Nd, ^{203}Tl, and ^{206}Pb. Samples #5 and #6 were spiked with 10^{-6} g of the aforementioned stable isotopes. The pH of each sample was adjusted to between pH 5.2 and 5.3. A column of Chelex 100 was prepared in ammonium form and each sample was passed through the column under gravity feed. After the collection of the trace elements by the resin, each column was washed with 5 g of water and 40 g of 1.0 M ammonium acetate. The column was again washed with 10 g of water. The column was then stripped using 7 mL 2.5 M HNO_3. Each of the acid effluents was collected in a Teflon beaker. Several drops of HCl were added to each beaker and the sample evaporated to dryness. The samples were treated in various ways and presented to Dr. P. J. Paulsen of the Analytical Spectrometry Section, the Analytical Chemistry Division in the Institute for Materials Research at the National Bureau of Standards. The spark source mass spectrometer utilized for analysis was a Consolidated Electrodynamics Corporation (CEC) Model 21-110 utilizing the photoplate technique. Sample #5 was analyzed directly after the elimination of the ammonium ion. Sample #6 was plated onto gold wire at 3.00 volts for twenty-four hours and then analyzed. Sample #4 was heated in a quartz crucible at 208-350 °C for twenty-four hours before analysis.

Instrumental Evaluation and Application Procedure Using Graphite Furnace Atomic Absorption Spectrometry

Portions of this research were done in conjunction with the Analytical Spectrometry Section, Analytical Chemistry Division of the National Bureau of Standards. The research in preconcentration and separation of trace transition elements from the sea water matrix was within the scope of ongoing trace analysis projects involving graphite furnace atomic absorption spectrometry (GFAAS). The usefulness of the final matrix for trace elemental samples of sea water was evaluated by Mr. T. C. Rains and Mr. T. J. Brady for compatibility with current high sensitivity atomic absorption equipment. The Perkin-Elmer Model 603 Atomic Absorption Spectrometer with heated graphite furnace with the AS-1 auto sampler was used. The accuracy of the preconcentration technique was evaluated by employing standard additions during sample preparation. To aid in the identification of the matrix effects of the major cations of sea water on the analysis of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn, the analysis of these elements was attempted from sea water directly and with the use of matrix modification as described by Ediger et al. (41). The amount of Na, K, Ca and Mg remaining with the trace metals was evaluated using ammonium acetate for the separation of these salts from the Chelex 100 resin. Analysis of the separation procedure using water and ammonium nitrate substituted for ammonium acetate was also accomplished. The homogeneous Chesapeake Bay sea water sample was used to evaluate the quantitation of the method for the transition metals. Four samples were untreated, while six samples of this sea water were spiked with µg quantities of the transition elements of interest: 50 µg Cd, 100 µg Co, 100 µg Cu, 200 µg Fe, 200 µg Mn, 200 µg Ni, 100 µg Pb, and 50 µg Zn. Four total process blanks of 100 mL each of sub-boiling distilled water were carried throughout the entire process to obtain a blank level for each element. These samples were then analyzed by GFAAS.

Alaskan sea water samples were prepared using the Chelex 100 resin column preconcentration and separation technique. Fifteen samples were analyzed in

triplicate, with one replicate of each sample spiked for Cd, Mn, Ni, and Pb. These samples were analyzed using the previously described GFAAS apparatus. Experimental conditions for the analysis by GFAAS of the artificial matrix of 2.5 M HNO_3 and residual NH_4^+ were developed and optimized for each element. They appear in Appendix D.

5. RESULTS AND DISCUSSION

SEPARATING AGENT SELECTION

Characteristics Desired in the Separation

In developing a separation technique there exist a number of objectives beyond the separation which determine the usefulness of the technique. The parameters developed and reagents utilized must not themselves become hindrances to the compatibility of the final sample and the applications intended to benefit from the separation.

These characteristics were considered essential to the successful utilization of a separation technique developed for the removal of Na, K, Ca and Mg from a Chelex 100 resin column:

—A reagent should be capable of selectively removing Na, K, Ca and Mg from a Chelex 100 resin column without removal of transition metals or disruption of the resin. This would be the most effective approach.

—The reagent itself must be innocuous to the instrumental applications desired. Thus, it would be desirable if the reagent could be completely eliminated from the final matrix containing the trace transition metals. This would be most satisfactory to all applications simultaneously.

—Contamination from the reagent should be minimal. A reagent which has ultrapurification procedures available is most desirable.

These characteristics serve as a starting place for the research.

Early Experiments

In recalling the selectivity series for cations chelated on Chelex 100 resin, the alkali and alkaline earth elements are the two most weakly held groups. Sodium and K are held many orders of magnitude more weakly and are the most easily removed. Calcium and Mg, however, are chelated with the same order of magnitude in selectivity as some of the transition metals (Appendix B). Manganese is the most weakly held transition metal, only 0.024 in comparison with 0.013 for Ca selectivity. Since some of the more weakly held transition elements may also be removed in an attempt to remove Ca and Mg, Mn is a logical choice to act as a model for the transition elements in early experiments with various separating agents.

Dilute nitric acid was evaluated as a possible separating agent. A 0.05 M solution of acid was passed through a column of Chelex 100 resin which had chelated 100 mL of sea water. Sodium and K were removed with 6 to 8 mL, but Ca and Mg were only slightly removed, with 2,000 µg of Ca remaining in the final volume when 2.5 M HNO_3 was used to strip the column. The hydrogen ion, although an efficient stripping agent for the Chelex 100 resin, has several drawbacks as a selective eluting agent. If the acid solution is too concentrated or too much is added, the pH of the entire column is lowered below pH 5.0 and all elements chelated are eluted from the column simultaneously. This totally defeats the purpose of the separation. Although nitric acid is useful as a stripping agent to totally remove the ionic content of a Chelex 100 resin column, its use as a selective eluting agent is limited to Na and K only, as described by Lee et al. (25).

The ammonium ion has many of the high purity and non-interfering characteristics of the hydrogen ion. (It is the preferred form of the resin for the concentration of transition metals, over that of the hydrogen ion, preventing low pH problems in the resin.) An initial experiment using ammonium nitrate was prepared to attempt the elution of Na, K, Ca and Mg from the resin. Ammonium nitrate (0.4 M) was placed on a Chelex 100 resin column after chelation of 100 mL of sea water at pH 6.0. After the collection of 10 mL of ammonium nitrate in 2 mL aliquots, the column was stripped with 2.5 M HNO_3. Analysis of the effluent by atomic absorption spectrometry (AAS) indicated a tenfold increase in the amount of Ca removed from the column during the ammonium nitrate fraction. The Na and K showed the same pattern of elution as with the dilute nitric acid, being eliminated in approximately 6 mL of the ammonium nitrate. However, the amount of Ca remaining in the 2.5 M HNO_3 was approximately 2,000 µg.

The Comparison of Ammonium Nitrate and Ammonium Acetate as Separating Agents

The preliminary experiment with ammonium nitrate indicated some Ca and Mg removal using the ammonium ion. Ammonium nitrate of high purity was prepared as previously described. The solution concentration was increased to 1.0 M. A second ammonium salt, ammonium acetate, was prepared, also at 1.0 M. The acetate form was chosen for two reasons. First, it forms metal complexes with Ca and Mg but does not form any extremely strong complexes with transition metals (56). Secondly, it buffers in the pH range 5.0-5.5 and serves to stabilize the critical pH during the elution. Because the effect of these ammonium salts on the transition metals chelated by the resin was not known, the sea water was spiked with 100 µg of Mn prior to chelation to allow detection in the final volume. The experimental results for ammonium nitrate as an eluting agent for Na and K appear in Table 1. Its effect on Ca and Mg is shown in Table 2. The evaluation of the transition metals is modeled by Mn in Table 3. The results of ammonium acetate for the elution of Na and K are given in Table 4 and its effects on Ca and Mg are grouped in Table 5. The elution of Mn is shown in Table 6.

Since the effluent was collected by weight (to prevent contamination) as previously described in the Procedure, it is necessary to identify the density of 1.0 M ammonium acetate to correct the weight to the volume. The density of 1.0 M ammonium acetate was therefore determined, using a pycnometer, to be

TABLE 1. THE GRADIENT ELUTION OF Na AND K FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM NITRATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Na			K		
			Concentration µg/mL	µg	Percentage	Concentration µg/mL	µg	Percentage
1	NH_4NO_3	4.91	510	2500	99.3	26.8	132	99.4
2	NH_4NO_3	9.70	3.9	18.7	0.7	0.15	0.7	0.6
3	NH_4NO_3	14.75	<0.1			<0.1		
4	NH_4NO_3	19.31	<0.1			<0.1		
5	NH_4NO_3	23.87	<0.1			<0.1		
6	NH_4NO_3	28.79	<0.1			<0.1		
7	NH_4NO_3	33.99	<0.1			<0.1		
8	NH_4NO_3	38.77	<0.1			<0.1		
9	NH_4NO_3	44.02	<0.1			<0.1		
10	NH_4NO_3	49.00	<0.1			<0.1		
11	H_2O	52.57	<0.1			<0.1		
12	HNO_3	58.91	<0.1			<0.1		
13	HNO_3	63.83	<0.1			<0.1		
14	HNO_3	68.52	<0.0			<0.1		
Total				2519	100.0		133.	100.0

TABLE 2. THE GRADIENT ELUTION OF Ca AND Mg FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM NITRATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

			Ca			Mg		
Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage	Concentration µg/mL	µg	Percentage
1	NH_4NO_3	4.91	341	1670	30.28	347	1700	36.98
2	NH_4NO_3	9.70	255	1220	22.12	299	1432	31.15
3	NH_4NO_3	14.75	70.0	354	6.42	96.3	486	10.57
4	NH_4NO_3	19.31	38.9	117	2.12	36.8	168	3.65
5	NH_4NO_3	23.87	25.5	126	2.29	23.6	116	2.52
6	NH_4NO_3	28.79	37.7	186	3.37	26.2	129	2.80
7	NH_4NO_3	33.99	33.7	175	3.17	21.7	113	2.46
8	NH_4NO_3	38.77	85.3	408	7.40	23.9	114	2.48
9	NH_4NO_3	44.02	46.1	242	4.39	22.5	118	2.57
10	NH_4NO_3	49.00	39.8	198	3.59	20.2	101	2.20
11	H_2O	52.57	81.0	289	5.24	10.6	37.8	0.82
12	HNO_3	58.91	83.6	530	9.61	13.0	82.4	1.80
13	HNO_3	63.83	<0.1			<0.1		
14	HNO_3	68.52	<0.1			<0.1		
TOTAL				5515	100.0		4597	100.0

TABLE 3. THE ELUTION OF Mn FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN SUBJECTED TO 1.0 M (pH 5.2) AMMONIUM NITRATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Mn		
			Concentration µg/mL	µg	Percentage
1	NH_4NO_3	4.91	<0.1		
2	NH_4NO_3	9.70	<0.1		
3	NH_4NO_3	14.75	<0.1		
4	NH_4NO_3	19.31	<0.1		
5	NH_4NO_3	23.87	<0.1		
6	NH_4NO_3	28.79	<0.1		
7	NH_4NO_3	33.99	<0.1		
8	NH_4NO_3	38.77	<0.1		
9	NH_4NO_3	44.02	<0.1		
10	NH_4NO_3	49.00	<0.1		
11	H_2O	52.57	<0.1		
12	HNO_3	58.91	17.0	108	100
13	HNO_3	63.83	<0.1		
14	HNO_3	68.52	<0.1		
TOTAL				108	100

TABLE 4. THE GRADIENT ELUTION OF Na AND K FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Na			K		
			Concentration µg/mL	µg	Percentage	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	4.92	510	2509	99.25	20.1	98.9	99.13
2	NH_4COOCH_3	9.62	3.94	18.5	0.73	0.18	0.87	0.87
3	NH_4COOCH_3	14.34	0.07	0.35	0.02	0.1		
4	NH_4COOCH_3	19.00	<0.1			<0.1		
5	NH_4COOCH_3	24.24	<0.1			<0.1		
6	NH_4COOCH_3	28.91	<0.1			<0.1		
7	NH_4COOCH_3	33.53	<0.1			<0.1		
8	NH_4COOCH_3	38.21	<0.1			<0.1		
9	NH_4COOCH_3	42.87	<0.1			<0.1		
10	NH_4COOCH_3	47.44	<0.1			<0.1		
11	H_2O	50.85	<0.1			<0.1		
12	HNO_3	56.52	<0.1			<0.1		
13	HNO_3	61.37	<0.1			<0.1		
14	HNO_3	65.87	<0.1			<0.1		
Total				2528	100.0		99.8	100.0

TABLE 5. THE GRADIENT ELUTION OF Ca AND Mg FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

			Ca			Mg		
Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	4.92	359	2258	55.2	695	3419	78.3
2	NH_4COOCH_3	9.62	294	1382	33.8	185	870	19.9
3	NH_4COOCH_3	14.34	85.5	407	9.9	15.6	74.3	1.7
4	NH_4COOCH_3	19.08	9.02	42.4	1.0	0.775	3.64	0.1
5	NH_4COOCH_3	24.24	0.819	4.23	0.1	<0.1		
6	NH_4COOCH_3	28.91	0.002	0.01	0.01	<0.1		
7	NH_4COOCH_3	33.53	<0.1			<0.1		
8	NH_4COOCH_3	38.21	<0.1			<0.1		
9	NH_4COOCH_3	42.82	<0.1			<0.1		
10	NH_4COOCH_3	47.44	<0.1			<0.1		
11	H_2O	50.85	<0.1			<0.1		
12	HNO_3	56.52	<0.1			<0.1		
13	HNO_3	61.37	<0.1			<0.1		
14	HNO_3	65.87	<0.1			<0.1		
TOTAL				4094	100.01		4367	100.0

TABLE 6. THE ELUTION OF Mn FROM A 100 mL SEA WATER SAMPLE PRECONCENTRATED ON A CHELEX 100 RESIN COLUMN SUBJECTED TO 1.0 M (pH 5.2) AMMONIUM NITRATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Mn		
			Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	4.92	<0.1		
2	NH_4COOCH_3	9.62	<0.1		
3	NH_4COOCH_3	14.34	<0.1		
4	NH_4COOCH_3	19.08	<0.1		
5	NH_4COOCH_3	24.24	<0.1		
6	NH_4COOCH_3	28.91	<0.1		
7	NH_4COOCH_3	33.53	<0.1		
8	NH_4COOCH_3	38.21	<0.1		
9	NH_4COOCH_3	42.87	<0.1		
10	NH_4COOCH_3	47.44	<0.1		
11	H_2O	50.85	<0.1		
12	HNO_3	56.52	16.0	91	100
13	HNO_3	61.37	<0.1		
14	HNO_3	65.87	<0.1		
TOTAL				91	100

1.0079 g/mL. The correction from weight to volume was found to be unnecessary for 1.0 M ammonium acetate. However, the 2.5 M nitric acid used in eluting the elements does require a correction using the density 1.08 g/mL (57).

A comparison of the separation performed with ammonium nitrate and ammonium acetate for Na and K can be made by evaluating Tables 1 and 6, respectively. It can be seen that both of these ammonium salts eliminate Na and K at an extremely rapid rate. Approximately 9 mL of either ammonium salt reduces the concentration of Na and K to <0.1 μg/mL. It is also evident that this is a complete separation, with neither element appearing in the 2.5 M HNO_3 used to strip the column. Figure 2 is a graphical representation of Tables 1 and 4.

In comparing the elution of Ca and Mg from the column with ammonium nitrate and ammonium acetate (Tables 2 and 5, respectively), a decidedly dissimilar set of results is observed.

The ammonium nitrate did remove a large portion of the Ca from the column. However, it was not a complete separation, even when using 50 mL of 1.0 M reagent. Ten percent of the Ca was eluted with 2.5 M HNO_3. This represents over 0.5 mg of Ca which would be present with the trace transition metals in this volume. The elution curve can be seen graphically represented in Figure 3. It appears to be a slow continuous process throughout the 50 mL of reagent, with an exception in the initial 10 mL. This first 10 mL of reagent also contains Ca from the residual resin volume. (Further discussion of this volume is necessary later in this Section.)

The ammonium acetate elution of Ca assumes an appearance much like that of a chromatographic peak, with much less tailing than when using ammonium nitrate. It is a more complete separation; a quantity of less than 0.1 μg/mL Ca remains in the 2.5 M HNO_3 fraction. Complete separation of Ca is accomplished by 30 mL of 1.0 M ammonium acetate. The ammonium acetate and ammonium nitrate separation of Ca can be compared graphically in Figure 3.

Both ammonium nitrate and ammonium acetate exhibit the removal of Mg in patterns similar to those of the Ca removal, with the exception of a reduced amount of Mg in the final volume of ammonium nitrate. These separations can be compared graphically in Figure 4.

Neither ammonium nitrate nor ammonium acetate produces the elution of Mn (Tables 3 and 6). However, 2.5 M HNO_3 completely strips the Mn from the column in less than 6 mL.

These findings support several conclusions. Since the nitrate ion is a neutral ion without an affinity for forming Ca and Mg complexes, the separation of Ca and Mg from the Chelex 100 resin using ammonium nitrate is primarily due to the ammonium ion. Using ammonium acetate, there appears to be assistance from the acetate ion in the separation of Ca and Mg, in addition to that of the ammonium ion. Therefore, ammonium acetate, rather than ammonium nitrate, emerges as the most efficient separating agent for the removal of Na, K, Ca and Mg from the Chelex 100 resin.

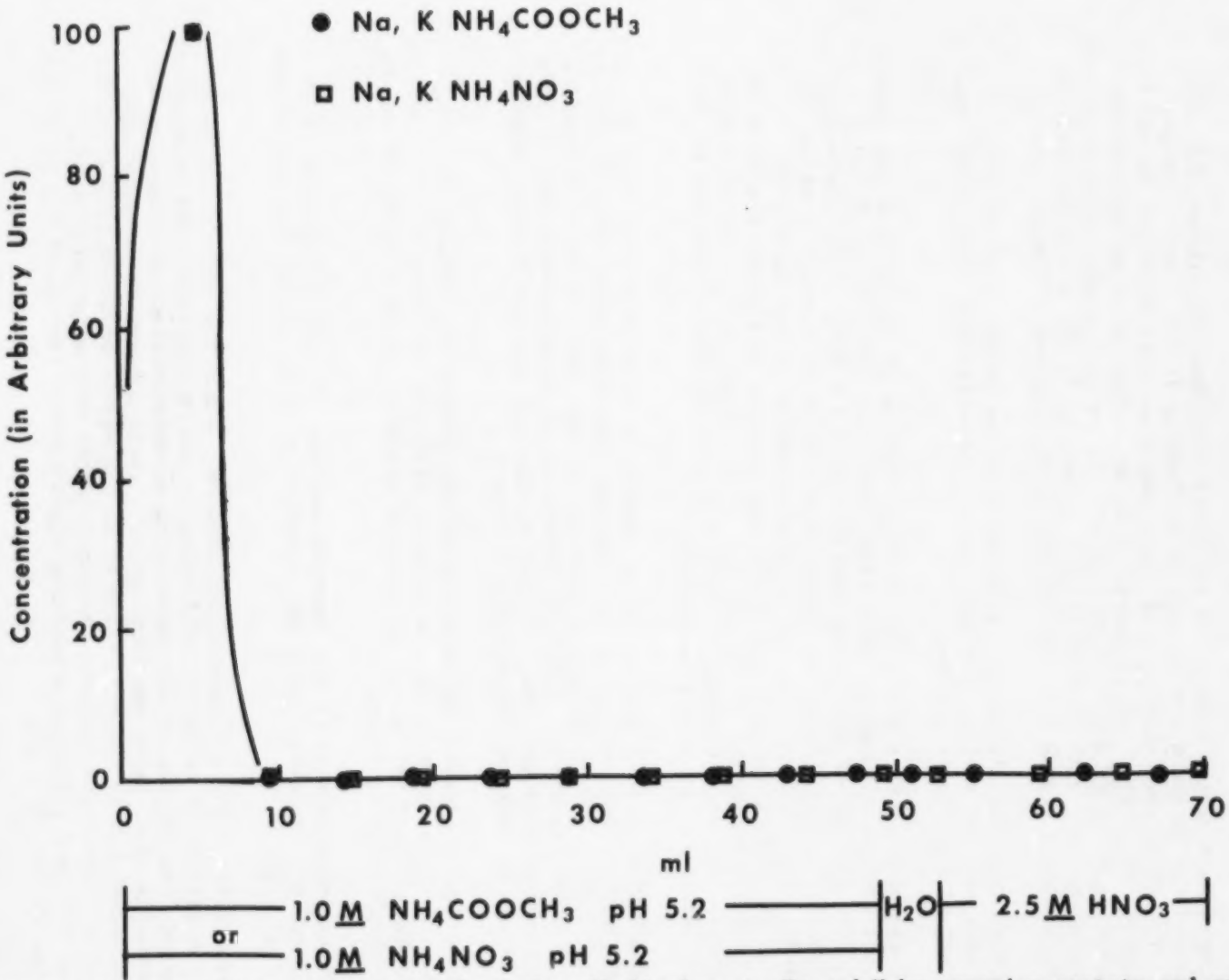


Figure 2. The comparison of the removal of the alkali elements Na and K by ammonium acetate and ammonium nitrate from a Chelex 100 resin column which has previously chelated 100 mL of sea water.

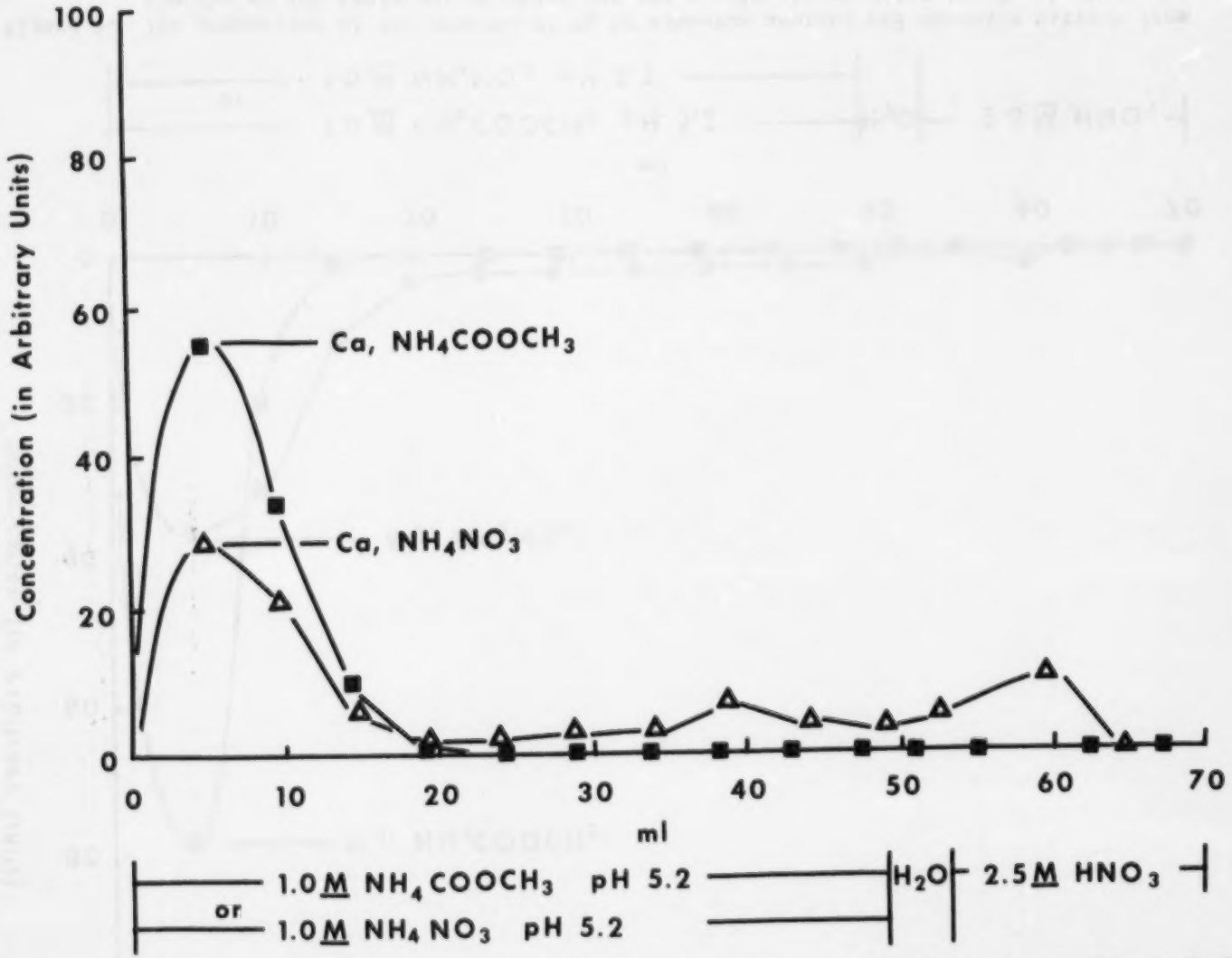


Figure 3. The comparison of the removal of Ca by ammonium acetate and ammonium nitrate from a Chelex 100 resin column which has previously chelated 100 mL of sea water.

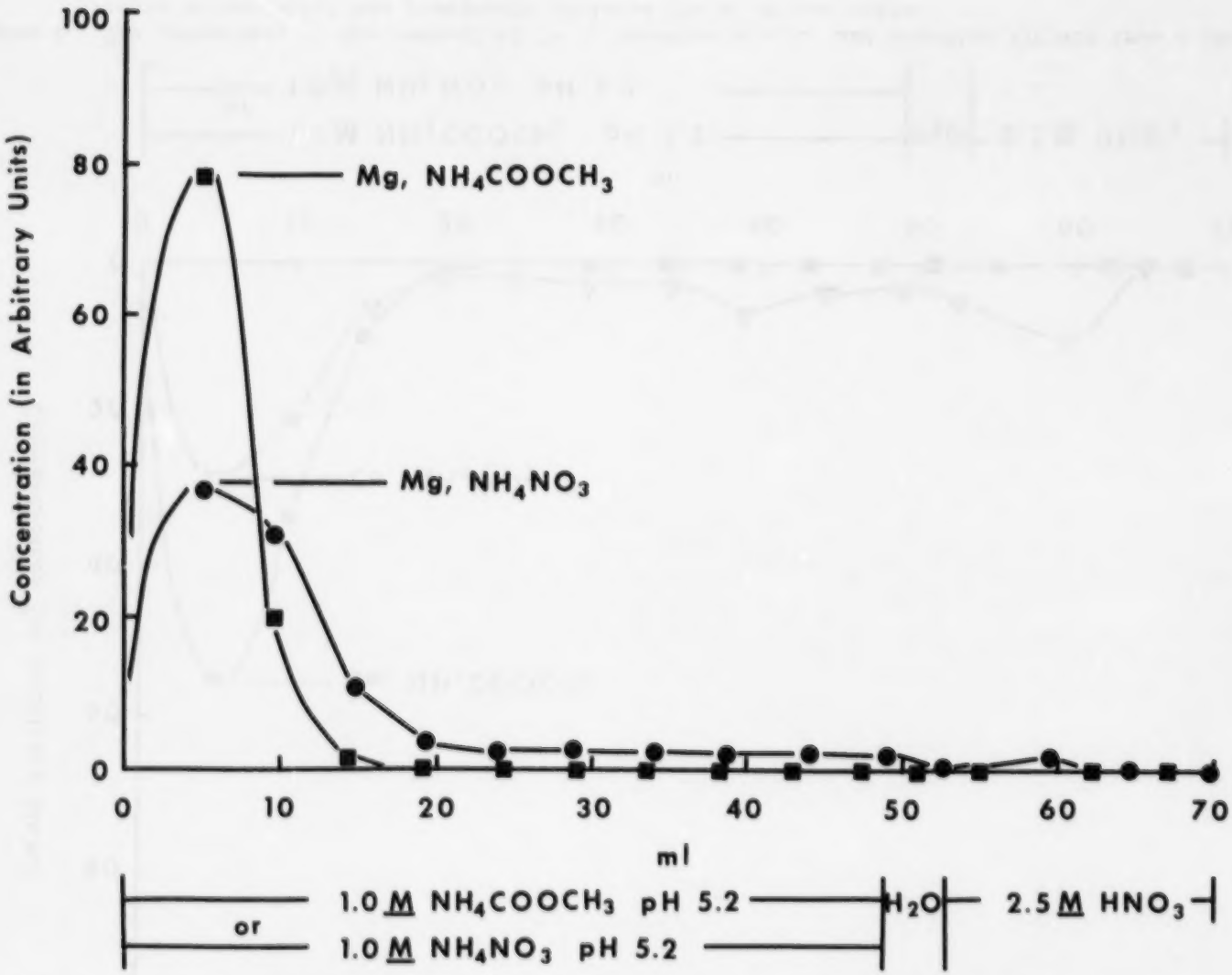


Figure 4. The comparison of the removal of Mg by ammonium acetate and ammonium nitrate from the Chelex 100 resin column which has previously chelated 100 mL of sea water.

CONCENTRATION AND SEPARATION PARAMETERS

Evaluation of the Influence of pH on the Separation

The pH of the ammonium nitrate and ammonium acetate used to separate the Na, K, Ca and Mg in the previous study was pH 5.2. Since the reduction of pH is also a method for removing ions from the resin, a reduction in the pH of the ammonium acetate could perhaps increase the efficiency of the separation, require less reagent to achieve the separation, and lower any blank that might be contributed by the ammonium acetate. A study of the influence of pH on the separation was initiated as described in the Procedure. The results have been graphed in Figure 5.

The Mn is eluted by the ammonium acetate at any pH below pH 5.0. The Mn overlaps the Ca peak from pH 3.0-4.5 and does not provide enough separation from the Ca to obtain a complete separation. The Ca and Mg are eluted very rapidly at pH 3.0, but from 3.5-5.0 they appear to change very little in placement of the elution or the tailing towards the Mn. To obtain a complete separation using ammonium acetate a pH of 5.0 or greater is necessary to prevent Mn from being stripped with the Ca and Mg. This permits the retention of Mn and presumably the other transition metals until stripped simultaneously in a small volume of 2.5 M HNO_3.

Parameters Fixed for Further Study

The parameters used in the ammonium acetate separation were standardized and repeated throughout all subsequent 100 mL sea water sample preparations. These conditions are given in the Procedure. A composite of Tables 4, 5, and 6 was constructed to provide Figure 6. This figure shows the conditions for the separation of the alkali and alkaline earth elements from a column of Chelex 100 resin. It also shows the elution of Na, K, Ca and Mg from a resin column that has preconcentrated a 100 mL sea water sample. The location of the transition metals was also influenced by preliminary qualitative studies of the radiochemical tracers ^{54}Mn, ^{109}Cd and ^{65}Zn.

The conditions specified in Tables 4 through 6 were repeated in each use of this technique hereafter to eliminate an alteration in procedure affecting the experimental results. Figure 6 can be viewed as a generalization of the separation treatment applied to any sea water sample introduced.

The use of the water prior to stripping of the column of the transition elements was not previously discussed due to its inconsequential effect on the separation. It was originally placed in the separation to prevent the buffering action of the residual ammonium acetate remaining on the column prior to stripping with 2.5 M nitric acid. However, some preliminary experiments utilizing the 2.5 M nitric acid concentrate by GFAAS indicated that the acetate ion caused suppression of the signal of some elements. As an organic, acetate was also not favorable for SSMS and was undesirable in the final volume. Therefore, the small water wash remained in the procedure to remove the acetate anion from the column. It is not chelated by the resin and is effectively removed with the residual aqueous volume prior to stripping of the column by 2.5 M nitric acid.

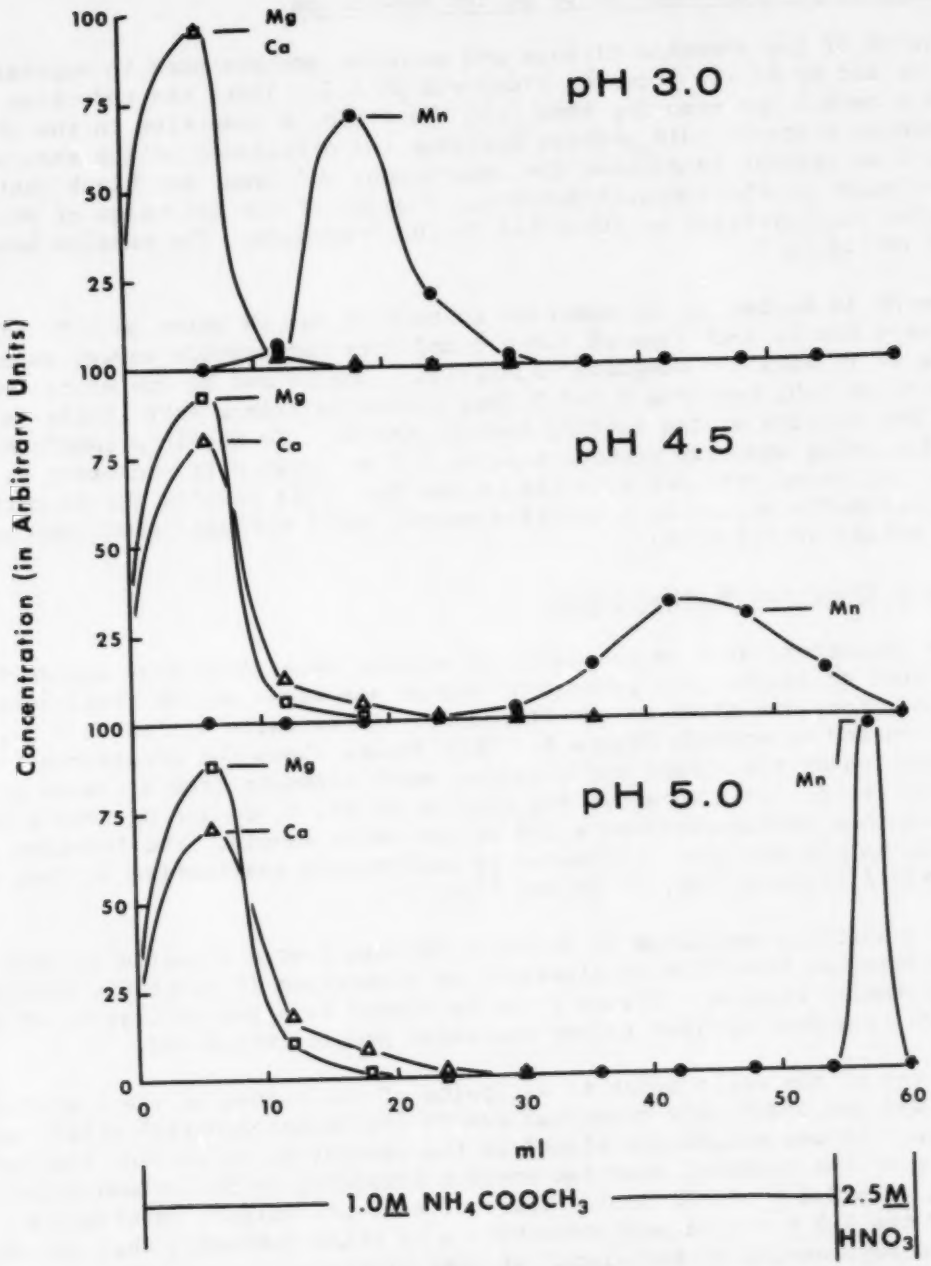


Figure 5. The elution of Ca, Mg and Mn from a preconcentrated 50 mL sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent at pH 3.0, 4.5, and 5.0 and 2.5 M nitric acid stripping agent.

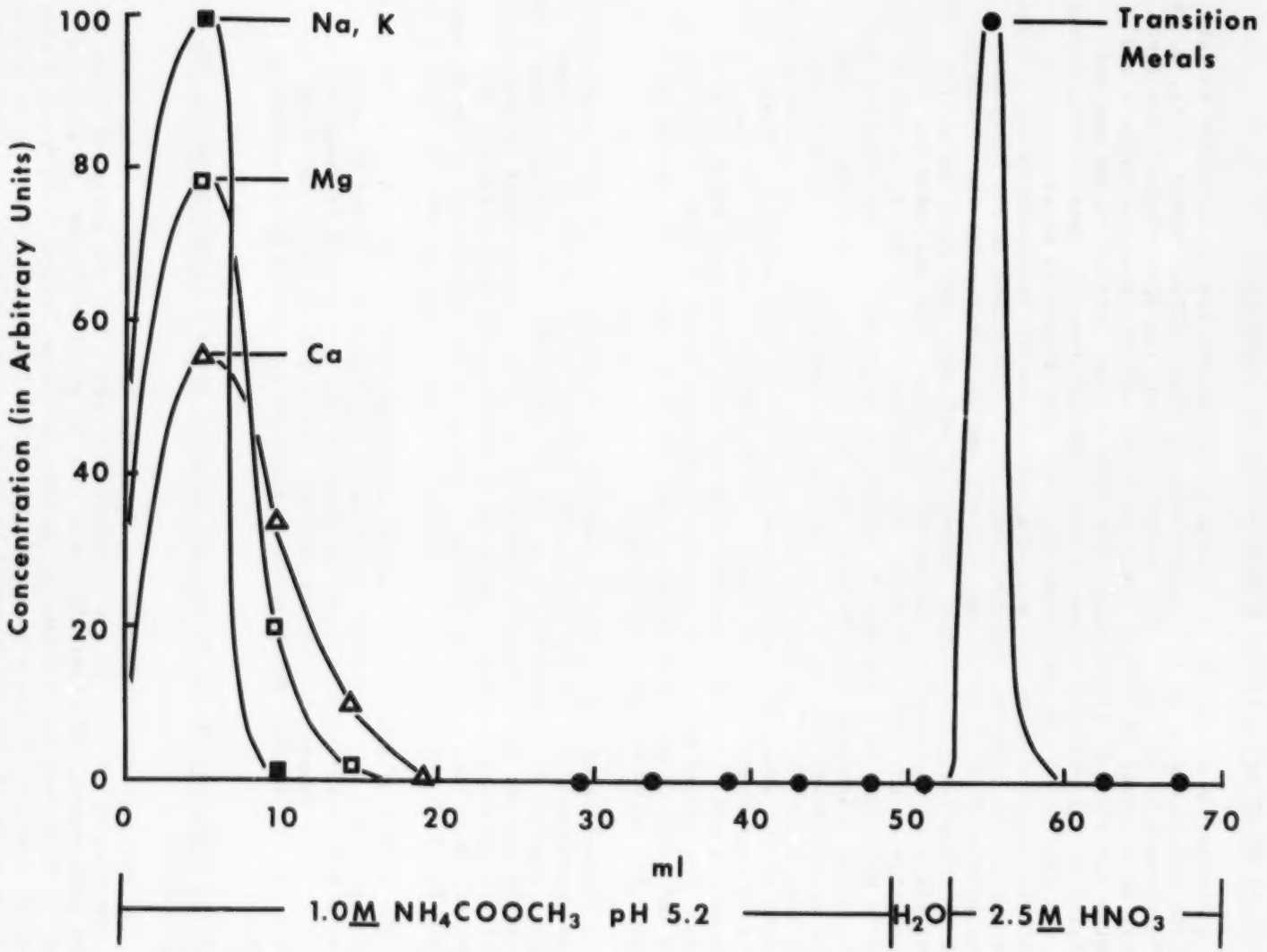


Figure 6. The elution of Na, K, Ca, and Mg from a preconcentrated 100 mL sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent and 2.5 M nitric acid stripping agent.

ASPECTS OF THE SEPARATION

The Use of Water as an Aid in Understanding the Separation

A discussion of the inital volume in the separation is necessary to gain a complete understanding of the separation. A large initial amount of Na, K, Ca and Mg appears in the separating reagent effluent (in approximately the first 10 mL), as observed in Tables 1 through 6 for both the ammonium nitrate and ammonium acetate. It was assumed that much of this initial volume was the expulsion of the residual volume in the resin and that the ions in this volume were not only being stripped chemically but also displaced physically. A sample was prepared using the standard conditions for preconcentrating a 100 mL sea water sample onto the standard resin column described in the Procedure. Approximately 50 mL of water (neutral pH) was substituted for the separating agent to investigate the initial removal of all four ions from the column within the first 10 mL volume of the separating agent. The sea water was analyzed concurrently as the study was done. The concentration of Na, K, Ca and Mg was found to be 6,200, 267, 283 and 742 µg/mL, respectively, in the original sea water sample (Chesapeake Bay Water). The ratio of the ions in the initial volume approximates the ionic ratio of these elements in the original sea water (see Table 7). The initial high amounts of the alkali and alkaline earth elements in the separating agents, ammonium acetate and ammonium nitrate, are partially contributed by this physical displacement. However, the ion exchanged Na and K is also almost completely removed in this first 10 mL volume. The amount remaining on the column after water elution is the amount of Na and K not displaced physically, but which is chemically removed by the ammonium ion using either ammonium salt.

A comparison of the Ca and Mg removed by physical displacement exhibits a similar phenomenon. They are physically removed in the first 10 mL volume; chemical elution also contributes to the Ca and Mg in this initial volume. The removal throughout the remainder of the 50 mL of separating agent is due almost totally to chemical removal by ammonium acetate or ammonium nitrate. The amount of these ions actually removed by water in the remaining volume is negligible for both the alkali and alkaline earth elements, as seen in Table 7.

The amount of alkali and alkaline earth elements in the 2.5 M nitric acid stripping agent, as illustrated in Table 7, indicates the amount of these elements that would be left with the trace transition metals if these salts were not first separated from the column: Na, 2,375; K, 74.7; Ca, 2,599 and Mg, 466 µg. It is this quantity which is actually removed by the ammonium acetate.

Selective Chelation of the Alkali and Alkaline Earth Salt Matrix During Preconcentration

The ratio to one another of Na, K, Ca and Mg removed by physical displacement was similar to that in the original sea water, but the ratio of these ions in the acid removal of chemically held ions is quite different, as shown in Table 7. This same information can be obtained from Tables 1, 2, 4 and 5 when the total quantity of the elements in the separating agent fractions is added. The ratio of Na, K, Ca and Mg could be reduced to roughly 620:2.7:2.8:7.4 for

TABLE 7. THE QUANTITY OF Na, K, Ca, AND Mg FOUND IN VARIOUS EFFLUENT FRACTIONS SUBSTITUTING WATER FOR A SEPARATING AGENT IN THE ELUTION FROM A PRECONCENTRATED 100 mL SAMPLE OF SEA WATER ON A CHELEX 100 RESIN COLUMN

Sample No.	Reagent	Cumulative Volume	Na Concentration µg/mL	Na µg	K Concentration µg/mL	K µg	Ca Concentration µg/mL	Ca µg	Mg Concentration µg/mL	Mg µg
1	H_2O	8.90	859	7,645	40	356	35.2	313.3	93.8	834.8
2	H_2O	18.68	0.788	7.71	0.138	1.35	0.030	0.29	0.011	0.11
3	H_2O	28.81	1.78	17.5	0.667	6.54	0.024	0.24	0.007	0.07
4	H_2O	38.31	1.01	9.92	0.875	8.59	0.019	0.19	0.002	0.02
5	H_2O	48.19	0.63	6.22	0.779	7.70	0.059	0.58	0.001	0.01
6	HNO_3	55.62	319.8	2,375	10.06	74.71	350.0	2,599	63.1	466.
7	HNO_3	60.36	-		0.17	.08	-		-	
8	HNO_3	65.05	<0.01		<0.01		0.31	1.4	0.027	0.13
Total				10,061		454.9		2,915		1,308

the natural sea water, while fraction #6 of Table 7 indicates a ratio of 24:0.7:26:4.7. This exhibits a noticeable reduction in the ratio of Na and an increase of Ca by an order of magnitude. This is due to the amount of each ion chemically held by the Chelex 100 resin. The Ca, although present in a smaller amount than Na, K, or Mg in the sea water, has been selectively chelated by the resin and actually occupies the most sites on a column of Chelex 100 resin after the chelation of a sea water sample of at least 100 mL.

This serves to illustrate that without the removal of Ca by a separating agent prior to stripping of the column, the matrix would be dominated by Ca. This becomes important when several of the instrumental techniques are considered. The significant instrumental ramifications of this observation will be discussed specifically in a following section, with reference to Graphite Furnace Atomic Absorption Spectrometry.

The amount of Na, K, Ca, and Mg remaining in the final 2.5 M nitric acid purging of a Chelex 100 resin column which has preconcentrated a 100 mL sea water sample and has been eluted with 50 mL of either water, ammonium nitrate or ammonium acetate, is presented for comparison in Table 8.

TABLE 8. THE AMOUNT OF Na, K, Ca, AND Mg REMAINING IN THE FINAL 2.5 M NITRIC ACID VOLUME AFTER ELUTION OF THE RESIN COLUMN WITH 40 mL OF WATER, AMMONIUM NITRATE OR AMMONIUM ACETATE. THE COLUMN HAD PREVIOUSLY PRECONCENTRATED A 100 mL SEA WATER SAMPLE

	Na µg	K µg	Ca µg	Mg µg
H_2O[a]	2,375	74.71	2,599	466
NH_4NO_3[b]	0.0	0.0	530	82.4
NH_4COOCH_3[c]	0.0	0.0	0.0	0.0

[a]The data for H_2O as the eluting agent was taken from Table 7.

[b]The data for NH_4NO_3 as the eluting agent was taken from Tables 1 and 2.

[c]The data for NH_4COOCH_3 as the eluting agent was taken from Tables 4 and 5.

PHYSICAL PARAMETERS AND EXTENSION OF THE SEPARATION TO LARGER VOLUMES

Chelex 100 resin is a dynamic resin and in the ammonium form at a pH of 7 to 14, the resin shrinks to approximately one half of its original volume when subjected to sea water at pH 5.0-5.5. Appendix E lists different resin volumes with different ionic forms (58). This shrinkage is acceptable for the original

3.2-3.4 mL of resin (200-400 mesh) used for a 100 mL sea water sample. The flow rate, influenced only by gravity, is approximately 0.8 mL/min. A 100 mL sample can be placed on the column in four 25 mL portions in approximately two hours, using the QS-S 25 mL column reservoir.

When the use of larger volumes is necessitated, these characteristics become intolerable. When the column is filled with resin to its 6 mL capacity, the flow rate is less than 0.3 mL/min and 1 liter of sample requires fifty hours to percolate through the resin. Therefore, the Teflon apparatus diagrammed in the Apparatus section was developed to surmount these physical characteristics. Employing this apparatus, a 1 liter sample was preconcentrated in sixteen hours, with only initial adjustment of the flow conditions. The flow rate of 1 mL/min was attained by simultaneously adjusting the Teflon valve and reservoir height to control the pressure, as described in the Procedure. The residual volume of the entire apparatus which may retain sample was determined to be less than 0.1 mL. Constructing the apparatus of Teflon in all areas where contact is made with the sample utilizes the non-wetting and non-contaminating characteristics of this fluorocarbon, which can be scrupulously cleaned in acid (47).

A further benefit of this apparatus is that during the preconcentration onto the resin, the sample and column are protected from contamination from the environment; the only entrance into the system is a microbore tube which can be filtered to exclude particulate contamination. In addition to the apparatus' required use with large volumes, it possesses desirable qualities for field or shipboard use.

Using the Teflon apparatus, a 1 liter sea water sample was adjusted to pH 5.0 and preconcentrated as described in the Procedure. The ammonium acetate effluent was collected in approximately 5 mL volumes as the Na, K, Ca and Mg were stripped from the resin. Table 9 is the quantitation of Na in the ammonium acetate elution from this column. Table 10 shows the K eluted in this effluent. The elution of Ca is presented in Table 11 and the Mg is presented in Table 12. These results were used to typify the separation of the alkali and alkaline earth elements using 1 liter samples and are graphed in Figure 7. (Radiotracer data, discussed later in this chapter, was used to justify the location and quantity of the transition metals in Figure 7).

The pattern of elution is identical to that of the 100 mL volumes. It is extended only to the amount equal to the increase in the total quantity of resin, which is approximately double. These results were used to establish the procedure for preconcentrating and separating the trace transition metals from 1 liter sea water samples. The procedure was not altered from that indicated in the Procedure for any additional tests to avoid procedural differences in the comparison of results.

TABLE 9. THE ELUTION OF Na FROM A PRECONCENTRATED 1 LITER SAMPLE OF SEA WATER ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	5.85	2200	12870	96.1
2	NH_4COOCH_3	11.75	90	531	3.9
3	NH_4COOCH_3	17.36	0.09	0.5	<0.01
4	NH_4COOCH_3	22.80	<0.1		
5	NH_4COOCH_3	28.87	<0.1		
6	NH_4COOCH_3	35.09	<0.1		
7	NH_4COOCH_3	40.97	<0.1		
8	NH_4COOCH_3	46.48	<0.1		
9	NH_4COOCH_3	52.43	<0.1		
10	NH_4COOCH_3	58.56	<0.1		
11	NH_4COOCH_3	65.25	<0.1		
12	NH_4COOCH_3	69.50	<0.1		
13	H_2O	78.01	<0.1		
14	H_2O	80.27	<0.1		
15	HNO_3	87.93	0.2	1.5	0.01
16	HNO_3	93.37	<0.1		
17	HNO_3	98.18	<0.1		
TOTAL				13393	100.00

TABLE 10. THE ELUTION OF K FROM A PRECONCENTRATED 1 LITER SAMPLE OF SEA WATER ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	5.85	80	468	94.4
2	NH_4COOCH_3	11.75	4.7	28	5.6
3	NH_4COOCH_3	17.36	<0.1		
4	NH_4COOCH_3	22.80	<0.1		
5	NH_4COOCH_3	28.87	<0.1		
6	NH_4COOCH_3	35.09	<0.1		
7	NH_4COOCH_3	40.97	<0.1		
8	NH_4COOCH_3	46.48	<0.1		
9	NH_4COOCH_3	52.43	<0.1		
10	NH_4COOCH_3	58.56	<0.1		
11	NH_4COOCH_3	65.25	<0.1		
12	NH_4COOCH_3	69.50	<0.1		
13	H_2O	78.01	<0.1		
14	H_2O	80.27	<0.1		
15	HNO_3	87.93	<0.1		
16	HNO_3	93.37	<0.1		
17	HNO_3	98.18	<0.1		
TOTAL				496	100.00

TABLE 11. THE GRADIENT ELUTION OF Ca FROM A PRECONCENTRATED 1 LITER SAMPLE OF SEA WATER ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	5.85	310	1812	32.8
2	NH_4COOCH_3	11.75	200	1180	21.4
3	NH_4COOCH_3	17.36	110	617	11.2
4	NH_4COOCH_3	22.80	93	505	9.1
5	NH_4COOCH_3	28.87	72	438	7.9
6	NH_4COOCH_3	35.09	60	373	6.8
7	NH_4COOCH_3	40.97	42	247	4.5
8	NH_4COOCH_3	46.48	27	149	2.7
9	NH_4COOCH_3	52.43	16	95	1.7
10	NH_4COOCH_3	58.56	14	85	1.6
11	NH_4COOCH_3	65.25	2.7	18	0.3
12	NH_4COOCH_3	69.50	1.1	4.7	<0.1
13	H_2O	78.01	0.1	0.9	<0.1
14	H_2O	80.27	<0.1		
15	HNO_3	87.93	0.4	3	<0.1
16	HNO_3	93.37	<0.1		
17	HNO_3	98.18	<0.1		
TOTAL				5527	100.0

TABLE 12. THE GRADIENT ELUTION OF Mg FROM A PRECONCENTRATED 1 LITER SAMPLE OF SEA WATER ON A CHELEX 100 RESIN COLUMN USING 1.0 M (pH 5.2) AMMONIUM ACETATE SEPARATING AGENT AND 2.5 M NITRIC ACID STRIPPING AGENT

Sample No.	Reagent	Cumulative Volume (mL)	Concentration µg/mL	µg	Percentage
1	NH_4COOCH_3	5.85	772	4512	48.7
2	NH_4COOCH_3	11.75	493	2910	31.4
3	NH_4COOCH_3	17.36	190	1066	11.5
4	NH_4COOCH_3	22.80	87	473	5.1
5	NH_4COOCH_3	28.87	35	213	2.3
6	NH_4COOCH_3	35.09	10	62	0.7
7	NH_4COOCH_3	40.97	2.2	13	0.2
8	NH_4COOCH_3	46.48	0.76	4.2	0.1
9	NH_4COOCH_3	52.43	0.31	1.8	<0.1
10	NH_4COOCH_3	58.56	0.15	0.9	<0.1
11	NH_4COOCH_3	65.25	0.08	0.5	<0.1
12	NH_4COOCH_3	69.50	0.02	0.1	<0.1
13	H_2O	78.01	0.01	0.1	<0.1
14	H_2O	80.27	0.01	<0.1	<0.1
15	HNO_3	87.93	0.20	1.5	<0.1
16	HNO_3	93.37	0.22	1.2	<0.1
17	HNO_3	98.18	0.04	0.2	<0.1
TOTAL				9259.5	100.0

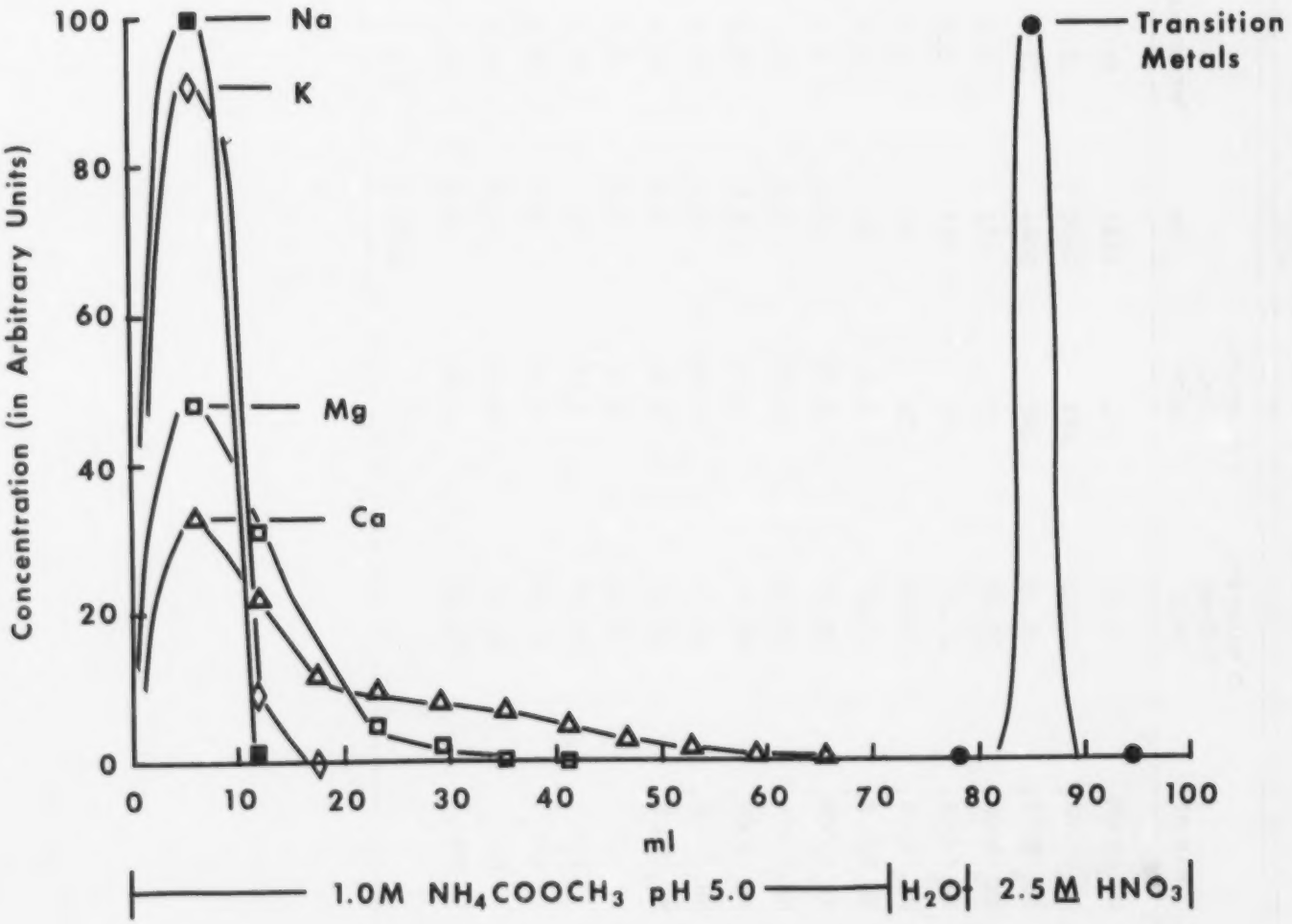


Figure 7. The elution of Na, K, Ca, and Mg from a preconcentrated 1 liter sample of sea water on a Chelex 100 resin column using 1.0 M ammonium acetate separating agent and 2.5 M nitric acid stripping agent.

QUANTITATIVENESS OF THE TOTAL RECOVERY OF SELECTED TRANSITION ELEMENTS USING RADIOCHEMICAL TRACERS

> "When developing analytical methods for the determination of trace elements in sea water, it is essential to check the efficiency of the proposed concentration and separation process. This can be done chemically by spiking stripped sea water samples with known amounts of the appropriate species of the element in question and then checking the recovery by physico-chemical methods. However, if a suitable radio-nuclide of the element is available the recovery can be determined more simply radiochemically (it is more important that the radio-nuclide added should have a high specific activity so that the amount of the inert form of the element added is kept to a minimum). In both the chemical and radiochemical methods of checking the separation process it is, of course, vital that the spike should be equilibrated with that present naturally in the sample" (3).

J. P. Riley has defined well the purpose of the tracer study on this analytical technique. Other researchers have used Chelex 100 resin to preconcentrate the trace transition elements from marine waters and have reported recoveries of >99 percent for many of these elements. When altering an analytical procedure the yield may also be altered with the introduction of the separation procedure. Although no evidence of losses has occurred, the process would be unusable until a quantitative study was done on the elements that are to be determined using this method. The alkali and alkaline earth elements have thus far been the primary target of analysis and only a model of the trend of the transition elements has been followed through Mn. The quantitative recovery of the transition elements was obtained using radiochemical tracers. This technique allows the most accuracy with the least alteration of the actual sample. Most of the radiotracers used were carrier-free sources of less than 1 µg total elemental content, allowing the minimum alteration of the actual elemental ratios and total quantities.

Due to their varying specific activities and elemental concentrations, a different amount of each tracer was used. The elemental tracers ^{109}Cd, ^{59}Fe, ^{210}Pb, ^{54}Mn and ^{65}Zn were carrier-free reagents. The elements ^{60}Co, ^{64}Cu and ^{65}Ni were made at the NBS reactor facility and had concentrations of 3.55, 2.29 and 3.52 µg/mL, respectively. The extreme sensitivity for these radiotracers allowed the measurements of extremely minute total spike additions.

The radiochemical isotopes behave identically in chemical reactions and can be treated as the normal stable isotopes in chemical consideration (59,60, 61). "Disturbing isotope effects arise only rarely" (61) and for these tracers chemical considerations are identical to the naturally occurring isotopes until the instant decay occurs and transformation of the radiochemical element into another element takes place. This transformation presents no problem as long as the decay state is a non-radioactive form, or at least one with a distinct

mode and energy of decay different enough from the element being detected that they can be distinguished (61).

Addition of the tracer to the sea water sample occurred just after the sea water sample was weighed for analysis and prior to manipulation of the pH. Both the sea water and the tracers were 0.5 N in HNO_3 (with the exception of ^{59}Fe in HCl). The pH was then adjusted to between pH 5.0-5.5, allowing the natural and tracer elemental species to be altered simultaneously if any alterations in form occurred. During this procedure a Teflon coated stirring bar (with remote magnetic stirrer) was substituted for the Teflon stirring rod used on the samples destined for instrumental analysis. This was done to promote the most homogeneous conditions in every aspect.

The data obtained during these radiochemical tracer experiments are given in Tables 13 and 14.

Counting each column fraction had two distinct advantages. First, the amount of tracer was not only quantified in the acid volume where it can be utilized by instrumentation (thus establishing useful recovery), but the presence of the tracer in other specific fractions could be identified and could pinpoint the exact source of the "loss" for the total final efficiency to the concentration onto, separation and stripping of the Chelex 100 resin column.

Secondly, the technique of counting all effluent from the column, resin and QS-Q column (physical column shell) offers a closed system that lends its application to counting of all the tracers used in this study, short and long lived, with a minimum of counting error introduced due to decay of the tracer. Counting the tracers before and after the experiment could not be done with the short lived tracers ^{64}Cu and ^{65}Ni without a large introduction of error from decay during the experiment. However, the counting of all fractions involved in any one separation was accomplished in forty minutes, eliminating the error due to rapid decay. Only the ^{65}Ni exhibited two fractions possessing detectable gamma radiation and thus eliminated any correction. The ^{65}Ni, having a half life of 2.5 hours, decayed beyond useful detection (essentially background) during the 1 liter experiment and gave no results for that application.

The ^{54}Mn in the 1 liter experiment also gave no results. This occurred because the "breakthrough" point was reached at approximately 500 mL of the initial loading of the sea water and therefore nullified the remaining results for ^{54}Mn. To quantitatively hold Mn from a full 1 liter volume of sea water a slightly larger volume of resin would be required. This did answer the question of whether Mn was a good model for the trace transition metals during the earlier work. It was the only transition element tested that reached its "breakthrough" point. Thus, under these conditions and in this matrix it is the most weakly held of the transition elements, and a good indication of the first of this group to appear from the column. No attempt was made to increase the volume of resin to concentrate the Mn from a 1 liter volume, which would give an excellent recovery on a longer column, judging from the quantitative recovery in the 100 mL experiment. The parameters were established to compare the recoveries under a set of identical conditions. If the procedure were altered, the continuity of the other work would have been reduced. It can be suggested that

TABLE 13. SUMMARY OF THE RADIO TRACER STUDY RESULTS SHOWING THE PERCENTAGE OF ELUTION IN EACH OF THE EFFLUENT FRACTIONS FOR THE 100 mL SAMPLES[a]

Element	Loss Percentage			Recovery Percentage
	Sea Water Effluent	Ammonium Acetate Effluent	Column and Resin	HNO_3 Effluent
^{109}Cd	<0.07	<0.07	<0.07	99.99 ± 0.064
	<0.06	<0.06	<0.06	99.99 ± 0.079
^{60}Co	0.30 ± 0.04	<0.03	0.16 ± 0.03	99.54 ± 0.30
^{64}Cu	0.03 ± 0.001	<0.002	0.03 ± 0.001	99.94 ± 0.036
	0.012 ± 0.0005	0.001 ± 0.0004	0.011 ± 0.0005	99.976 ± 0.024
^{59}Fe[a]	5.3	0.25	0.22	94.23
	7.21	0.57	0.20	92.02
^{54}Mn	<0.048	<0.02	0.01 ± 0.006	99.99 ± 0.11
^{65}Ni	0.08 ± 0.007	<0.006	<0.06	99.92 ± 0.099
	0.09 ± 0.004	<0.003	<0.003	99.91 ± 0.067
^{210}Pb	1.42 ± 0.13	<0.01	0.18 ± 0.13	98.40 ± 0.48
^{65}Zn	0.020 ± 0.008	<0.03	<0.02	99.98 ± 0.11
	0.059 ± 0.006	<0.02	<0.02	99.94 ± 0.087

[a]The upper error limits have been used where zero was obtained based on one standard deviation in the computation of the error.

[b]The error on this determination is reserved and described in the text.

TABLE 14. SUMMARY OF THE RADIO TRACER STUDY RESULTS SHOWING THE PERCENTAGE OF ELUTION IN EACH OF THE EFFLUENT FRACTIONS FOR THE 1 LITER SAMPLES[a]

Element	Loss Percentage			Recovery Percentage
	Sea Water Effluent	Ammonium Acetate Effluent	Column and Resin	Nitric Acid Effluent
^{109}Cd	<0.024	<0.06	<0.012	99.99 ± 0.063
^{60}Co	1.74 ± 0.005	0.03 ± 0.01	0.89 ± 0.02	97.34 ± 0.17
^{64}Cu	0.17 ± 0.006	<0.007	0.01 ± 0.003	99.82 ± 0.069
^{59}Fe[b]	6.22	0.90	0.31	92.57
^{54}Mn[c]	-	-	-	-
^{65}Ni[d]	-	-	-	-
^{210}Pb	2.13 ± 0.14	<0.056	0.11 ± 0.081	97.76 ± 0.39
^{65}Zn[e]	<0.01	<0.03	<0.04	99.99 ± 0.12 100.1 ± 0.12

[a]The upper error limits have been used when zero was obtained based on one standard deviation in the computation of the error.
[b]The error on this determination is reserved and discussed in the text.
[c]^{54}Mn exceeded the capacity of the column at 500 mL and was not evaluated.
[d]^{65}Ni decayed below useful levels and yielded no data for the 1 liter experiment.
[e]^{65}Zn was determined using two different techniques as discussed in the text.

a larger column for 1 liter samples would collect Mn quantitatively. In volumes over 1 liter, other elements may exhibit phenomena similar to the Mn if the column is kept at its present size. Thus, a 1 liter limit is suggested for the larger sea water volumes.

The technique of counting each fraction was checked using ^{65}Zn by another counting technique. The ^{65}Zn was placed in 250 mL of the sea water and counted prior to manipulation; the 250 mL was added to 750 mL of sea water and the usual procedure was followed. The count (corrected counts in ten minutes) prior to manipulation was 742,152. The corrected count obtained after adjustment of the acid fraction to 250 mL, was 743,179 corrected counts in ten minutes. Done in the same manner as the other tracer studies, the recovery in the acid fraction was calculated to be 99.99 ± 0.12, when counted before and after manipulation, 100.1 ± 0.12 was calculated as the recovery. This indicates that losses which were not accounted for were not encountered and act as a check on the technique.

Using Table 13 for the results of the 100 mL sample volumes and Table 14 for the results of the larger 1 liter sea water volumes, the results for all the elements tested and all the fractions can be compared. Of the elements tested, none (with the exception of ^{59}Fe) showed any significant amount of the respective elements in the ammonium acetate fraction. This fraction is the total additional manipulation of separation added to the established procedure. The remaining volumes represent only the concentration of the transition elements by the column, the removal of the elements from the column and the resin and column remaining after removal. This effectively demonstrates that the separation of Na, K, Ca and Mg is obtained with no cost to the total overall efficiency, beyond concentration and elution from the column. The elements of interest are not sacrificed within detectable limits at the expense of the addition of the separation step.

The losses that occurred with ^{59}Fe are synonymous with other problems of Fe. The small amount of loss in the ammonium acetate fraction can be explained by residual loss from the previous sea water fraction which is still present in the residual volume. At least some contribution from this factor is certain since there is no wash between the sea water and the start of the ammonium acetate. The non-quantitative retention of Fe and Co on Chelex 100 was a major part of the paper by Callahan et al. (12). They reported that both Fe^{+3} and Co^{+3} were not quantitatively retained by Chelex 100, and tried several reducing agents on these ions to reduce and stabilize their total complement in sea water to the +2 state. The Co was found to be naturally retained, 97-98 percent on the Chelex 100 resin from sea water. This high retention rate for Co was attributed to the fact that approximately 96 percent of the Co in sea water naturally exists as Co^{+3} and the remainder exists as Co^{+3}. The Fe exhibited these same phenomena, but the recovery was usually only 92-95 percent. No data on the effective species ratios were given, but Fe is easily oxidized by air from Fe^{+2} to Fe^{+3}. A small difference in the general amount of stirring could then alter this Fe^{+2}/Fe^{+3} ratio and cause a different amount of the Fe to be initially absorbed onto the Chelex 100 resin column. The error limits on Fe were therefore withheld from the final summary. The error as calculated is a function of radiological counting uncertainty, not an experimental chemical alteration, and therefore does not apply in this case. For the other elements

(Ca, Mg, Cd, Co, Cu, Mn, Ni, Pb and Zn) the +2 state is the normal species in sea water (1).

The error limits represent very conservative limits for the data. The equation described in the Procedure was used and takes into account not only the error in the sample count, but also the background count. Several of the experiments were repeated to verify the results. The repeated experiments usually used a different amount of tracer (usually at least double) to alter this parameter, which should not alter the results if the values are true. In each case (with the exception of ^{59}Fe previously discussed) the uncertainty in the recovery was more than adequate to encompass both determinations. Table 13 contains these comparisons.

In Tables 13 and 14 the zero, negative and negligible counts were replaced by the unit greater than the upper detection limit. The determination was made that the error was approximately one order of magnitude more significant than the negligible counts and that this error bracketing the zero numbers was a more significant number and represented an upper limit more meaningful than the use of zero or ND (Not Detected) (55).

The use of both 100 mL and 1 liter samples in this quantitative study was intended to establish quantitative boundaries that would encompass a wide range of sample volumes. The 100 mL sample size was the lower or smaller limit and the 1 liter sample was the larger or upper limit. This was set up to establish a flexible range of sample volumes from 100 to 1,000 mL which had already been quantified. Any volume in between these two extremes would fall quantitatively between the recoveries obtained. This range is intended to encompass a large range of sample concentrations and instrumental needs which change with changing samples and instrumentation.

EVALUATION OF THE COMPATIBILITY OF THE CHELEX 100 CONCENTRATION AND SEPARATION WITH SPARK SOURCE MASS SPECTROMETRY

The spark source mass spectrometric (SSMS) technique has several unique features which make it a desirable technique for use in conjunction with multi-elemental samples of aqueous origin. It possesses the unique benefit of being both multielemental and independent of recovery for analytical measurement. The quantitative results are obtained from the isotopic ratio measurements of stable isotopes of the elements. The technique uses the addition of enriched stable isotopes, high in the lower naturally occurring abundance isotopes of the element of interest. Equilibration of these added spikes (enriched isotopes) and the naturally occurring isotopes of the elements in the sample prior to manipulation is of primary importance to the analytical determination. The quantitative determination is based only on the ratio of spike isotope to natural isotope determined using the mass spectrometer. Thus, the sample is independent of losses of both spike and natural isotope of an element after equilibration as the analysis depends on a ratio and not on the total amount. However, enough of the sample and spike is required to determine this ratio and prevent the blank from becoming significant. The technique will yield analysis if approximately 10^{-7} g of the element is present. This amount will yield a confidence of approximately five percent in the actual analysis.

The isotopic ratio measurements require a sample free of organic matter and low in other matrix ions. The organic ions fragment with unit charges and superimpose values on the elemental masses, causing uncertain results. The matrix ions, if they are in quantities high above those of the elements of interest, can dilute the sample and also undergo polymerization into multielemental forms possessing charges. The alkali and alkaline earth elements are especially notorious for this and join in many molecular combinations possessing charges (36).

Sea water samples were spiked with a multielemental isotopic spike; its elemental components appear in the Procedure. The samples were equilibrated, adjusted to pH 5.0-5.5, concentrated on a Chelex 100 resin column, separated from Na, K, Ca and Mg using ammonium acetate, and stripped from the column as described in the Procedure. A few drops of hydrochloric acid were added to the nitric acid fraction containing the spiked isotopes and the natural elements, and the solution warmed gently and then taken to dryness. This removed the residual ammonium ion (62). A sample was examined by the Analytical Spectrometry Group of the Inorganic Analytical Research Division at the National Bureau of Standards, for analysis by SSMS. The investigation showed interference of organic molecular origin. These organics were presumed to originate during the acid elution from the Chelex 100 resin and were probably break-down products from the resin. Sample #6 was plated onto gold wires (to eliminate any organic molecules) as described in the Procedure, and both the anode and cathode were analyzed by SSMS. A second technique was also utilized to eliminate the organic material. Sample #4 was heated with nitric acid in a quartz crucible at 208-250 °C for twenty-four hours and was then analyzed.

The samples were then checked for "good measurable signal" which would lead to routine analysis for the element. The spiked isotope was used as an indicator of measurability if the normal isotopes were below detectable limits. The following elements were reported to have "good measurable signal" for sample #4: Pb, Nd, Tc, Cd, Sn, Ga, Zn, Cu, Ni, Fe, Cr, and Ti. The following elements were reported to have "good measurable signal" for sample #6: Pb, Nd, Cd, Sn, Ag, Ga, Zn, Cu, Ni, Fe, Cr (36).

Neither the organic nor matrix problems presented any difficulty in measurement when these techniques were used. The compatibility of the Chelex 100 resin concentration with the separation steps added, was confirmed. The addition of a post column step to eliminate any resin degradation products was necessary, but proved to be a problem which was quickly solved. The actual analysis of the elements was not pursued due to the amount of time and expense required to quantify the ratios from the photoplates. The evaluation of the technique as applicable for the instrument was achievable without complete quantitation of the sample due to the nature of the instrumental technique.

EVALUATION AND PRACTICAL USE OF THE CHELEX 100 SAMPLE PREPARATION METHOD UTILIZING GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY ANALYSIS

Unlike the SSMS, the graphite furnace atomic absorption spectrometry (GFAAS) cannot be qualitatively evaluated for its useful compatibility with the preconcentration separation technique. Only through the quantitative achieve-

ment of analysis could the compatibility of the sample preparation and instrumental GFAAS analysis technique be determined.

The GFAAS technique has a distinct advantage in its great sensitivity for elemental analysis. It also has a disadvantage; it is highly susceptable to matrix interelement effects.

The evaluation and analytical determinations made using the GFAAS were done in cooperation with the Analytical Spectrometry Group of the Inorganic Analytical Research Division of the National Bureau of Standards. The analysis utilized the Perkin-Elmer Model 603 Graphite Furnace Atomic Absorption Spectrometer.

Effects of Direct Injection of Sea Water Into the Electrothermal Device

From the detection limits published in the literature for GFAAS, it could be assumed that several of the heavy metals in sea water could be determined by direct injection of the sample into the electrothermal device. However, in reality this has not been proven to be true unless the samples are taken from heavily polluted areas. A sample from the Chesapeake Bay sea water samples was analyzed for Cd, Co, Cu, Mn, Ni, Pb, and Zn by direct injection into the graphite furnace by AAS. Only Pb and Ni produced absorption signals of any analytical value. The other elements could not be detected. This is due in part to the highly depressing effect of the matrix on the analyte signal, which can vary by a factor of two to ten, depending upon the analyte. Also, when the sample is evaporated, a small amount of solution may be trapped in the salt crystal lattice, which could result in losses due to splattering during the atomization cycle.

The absorbances obtained for Pb and Ni were very erratic due to the smoke produced during atomization. Ediger et al. (41) used matrix modification with ammonium nitrate to assist in the removal of sodium chloride; however, the method of standard addition was necessary to correct for interferences. In applying their method of matrix modification to the Chesapeake Bay sample, Cd, Co, Cu, Fe, Mn and Zn were still not detected (43).

Suppression Study of Ca on Mn and of Reagent Effects on Other Transition Metals

Because Ca is concentrated by Chelex 100 resin, a study was done of Ca suppression on the Mn signal using GFAAS. Standards were prepared containing 30 µg/mL Mn and varying amounts of Ca were added; 2 mg/mL, 1 mg/mL, 500 µg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL. Complete suppression of the Mn signal was observed for 2 mg/mL through 100 µg/mL. At 10 µg/mL approximately 33 percent of the signal was observed. With 1 µg/mL Ca the normal signal for 30 µg/mL Mn was observed. If the solution was one percent in HNO_3 also, the pattern changed drastically. The 1 and 2 mg/mL Ca additions produced enhancement of 12 percent in the Mn signal, and the Ca concentrations of 500 µg/mL and less gave normal Mn signals.

These tests showed the extreme effects that just one of the alkaline earth elements produces if remaining in the sample.

When the preliminary separation products using ammonium nitrate as the separating agent were placed in the graphite furnace, results similar to those for 500 µg/mL of Ca were observed. The sample was not compatible with the instrument with the residual Ca and Mg present at these levels.

The reagents used in the separation which could cause suppression or enhancement of the signal were checked using each element to be analyzed (Cd, Cu, Co, Fe, Mn, Ni, Pb and Zn). The nitric acid caused no significant enhancements or suppressions of the elements of interest. The nitric acid was also added to the standard solutions when they were prepared to keep the elements in solution.

The acetate ion (using ammonium acetate) was also evaluated in the initial experiments and found to cause some small suppressions or enhancement of the absorbance signal with several of the elements. It tended to enhance Cu, Pb and Co signal between two and five percent, and to suppress Cd and Fe between 15 and 50 percent, respectively. This problem was easily eliminated early in the development of the separation technique by the addition of a 5-10 mL water wash prior to stripping with nitric acid. This volume effectively removed the residual acetate which does not ion exchange or chelate onto Chelex 100 resin.

In addition to the suppression experiments, the nitric acid and ammonium acetate were checked for contamination of these reagents with the analytes of interest. Any level of contamination was below the detection limits of the GFAAS.

GFAAS Determination of Trace Elements in Sea Water

Evaluation of the preconcentration and separation methods was accomplished using GFAAS.

Prior to the preparation of the samples the density of the homogeneous sea water sample was determined. This enabled the samples to be weighed directly into a Teflon beaker without utilization of volumetric ware. The density was determined to be 1.0135 g/mL.

Six 100 mL samples of the Chesapeake Bay water were spiked with varying amounts of the elements of interest which are given in the Procedure and were used to check the recovery by GFAAS. Four samples were not spiked and all samples were processed as described in the Procedure. To determine the total blank, four blank columns were carried through the entire process of preparation, concentration, separation and stripping, using 2.5 M HNO_3. The 8-10 mL of 2.5 M HNO_3 effluent collected was analyzed for the trace elements of interest by GFAAS. Table 15 shows the results of these analyses. The instrumental conditions for drying, charring and atomizing appear in Appendix D. The conditions were optimized for each analyte to obtain the maximum sensitivity and precision with the minimum of interferences. As a check for chemical interferences, each sample was tested by the single standard addition method (63) and no chemical interferences were encountered. Complete recovery of the trace elements of interest from spiked sea water samples was obtained, as shown in Table 15.

TABLE 15. THE CONCENTRATION OF Cd, Co, Cu, Fe, Mn, Ni, Pb, AND Zn FOUND IN TEN IDENTICAL 100 mL SEA WATER SAMPLES FROM THE CHESAPEAKE BAY AS DETERMINED BY GFAAS AFTER CHELEX 100 CONCENTRATION AND SEPARATION

Sample No.[a,b]	CONCENTRATION, ng/mL							
	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
1	0.05	<0.1	1.9	2.1	2.1	1.3	0.5	4.5
2	0.06	<0.1	1.9	1.9	2.2	1.0	0.4	4.4
3	0.03	<0.1	1.8	2.5	2.2	1.2	0.7	4.4
4	0.03	<0.1	1.8	2.4	2.1	1.5	0.3	4.2
5	0.02	<0.1	1.8	1.4	2.2	1.1	0.4	4.1
6	0.05	<0.1	2.0	1.5	2.2	1.0	0.5	4.2
7	0.04	<0.1	2.0	1.6	2.0	1.2	0.2	4.5
8	0.05	<0.1	1.9	1.9	2.1	1.2	0.2	4.7
9	0.05	<0.1	3.1	2.1	2.0	1.2	0.6	4.9
10	0.05	<0.1	2.1	2.8	2.1	1.1	-[c]	4.9
11	<0.01	<0.1	<0.1	0.1	<0.1	<0.1	<0.2	<0.05
12	<0.01	<0.1	<0.1	0.3	<0.1	<0.1	<0.2	<0.05
13	<0.01	<0.1	<0.3	0.1	<0.1	<0.1	<0.2	<0.05
14	<0.01	<0.1	<0.1	0.1	<0.1	<0.1	<0.2	<0.05

[a]Samples 1-10 were taken from one homogeneous sample; the sea water samples 1-6 were spiked with the elements of interest, each containing ng/mL: Cd, 0.5; CO, 1.0, Cu, 1.0; Fe, 2.0; Mn, 2.0; Ni, 2.0; Pb, 10; Zn, 0.5. Samples 11-14 were blanks.

[b]Samples 1-6 are shown after subtraction of the spike.

[c]Contaminated.

Cobalt was not detected in the unspiked 100 mL sample. To obtain an analytical value for Co in the Chesapeake Bay samples, it would be necessary to concentrate and separate a 1 liter sample.

Some difficulty was encountered in the GFAAS determination of Fe. Iron is known to form carbides in the graphite furnace, which produce erratic results. Also, a high reagent blank was obtained. This could have been introduced by the environment at the time of analysis. Although the samples were prepared in a particulate-free environment, the analysis by GFAAS was carried out in a laboratory module constructed mainly of iron and steel.

The Pb values are close to the detection limits using a 100 mL sample. With a 0.5-1 liter sample the precision of the Pb analysis could be improved.

The largest spread between spiked and unspiked sea water samples was in the Zn analysis. This was due to the low concentration of Zn added to the spiked samples. Since Zn is so sensitive by GFAAS, a 1:10 dilution of the 2.5 M HNO_3 effluent had to be made. As a result, the original spike added to the sea water samples was too low, producing a slightly low recovery for the spiked samples.

After the samples had been analyzed by GFAAS for the elements of interest, samples #3, #8 and #13 were checked for Na, K, Ca and Mg content to verify the amounts in a typical analysis which exhibited no chemical interferences. Table 16 shows the results of this analysis.

TABLE 16. THE CONCENTRATION of Na, K, Ca AND Mg DETERMINED IN THE 2.5 M HNO_3 EFFLUENT OF THE CHESAPEAKE BAY SAMPLES AFTER ANALYSIS OF THE EIGHT TRANSITION ELEMENTS; SAMPLE 3 WAS SPIKED, SAMPLE 8 WAS UNSPIKED AND SAMPLE 13 WAS A BLANK

Sample No.	Concentration, µg/mL			
	Na	K	Ca	Mg
3	0.68	1.1	0.13	<0.05
8	1.47	2.7	0.36	0.06
13	0.12	<0.01	<0.01	<0.05

Other sea water samples were then analyzed using the Chelex 100 separation method and GFAAS analysis. These samples were sea water samples from Glacier Bay, Alaska. They possessed two characteristics distinguishing them from the Chesapeake Bay samples. First, they were more representative of open ocean water, with the average salinity being somewhat higher than in the Chesapeake Bay samples. Secondly, they were collected for their pristine character. These samples should approach a baseline on the concentration levels in uncontaminated oceanic samples. The analysis of these samples is given in Table 17. (An interesting discovery about frozen unacidified samples was made during the course of these analyses, but is not relevant to the continuity of the text at this point and has been placed in Appendix F.) The samples were indeed lower

TABLE 17. THE CONCENTRATION OF Cd, Mn, Ni, AND Pb FOUND IN FIFTEEN DIFFERENT SEA WATER SAMPLES OF 100 mL EACH, TAKEN FROM GLACIER BAY, ALASKA AS DETERMINED BY GFAAS AFTER CHELEX 100 RESIN COLUMN CONCENTRATION AND SEPARATION

Sample	Sample[a] Treatment	Original pH	Concentration ng/mL			
			Cd	Mn	Ni	Pb
NBS 500a	Fc, UA	7.3	<0.01	0.45	1.2	0.18
			<0.01	0.37	1.7	0.17
NBS 500b	UF, UA	7.2	<0.01	0.59	0.90	<0.02
			<0.01	0.67	0.70	<0.02
NBS 501a	Fc, UA	8.3	<0.01	0.40	0.49	<0.02
			<0.01	0.46	0.53	<0.02
NBS 501b	UF, UA	8.2	<0.01	0.71	0.66	<0.02
			<0.01	0.64	0.71	<0.02
NBS 502	UF, A	0.4	<0.01	1.3	0.45	4.5
			<0.01	1.4	0.59	5.8
NBS 503	UF, A	1.3	<0.01	1.4	0.41	<0.02
			<0.01	1.3	0.45	<0.02
Alaska 1a	F, A	3.5	0.11	0.05	0.24	0.02
			0.10	0.06	0.32	0.05
Alaska 1b	UF, A	3.6	0.12	0.02	0.49	<0.02
			0.13	0.02	0.40	<0.02
Alaska 2a	F, A	5.2	0.05	0.03	0.27	0.08
			0.06	0.03	0.29	<0.02
Alaska 2b	UF, A	6.5	0.11	<0.02	0.36	<0.02
			0.12	<0.02	0.38	<0.02
Alaska 3a	F, A	6.6	0.10	<0.02	1.7	<0.02
			0.14	<0.02	0.8	<0.02
Alaska 3b	UF, A	5.4	0.13	<0.02	0.90	<0.02
			0.12	<0.02	0.78	<0.02
Alaska 4a	F, A	5.1	0.07	0.22	0.36	0.20
			0.13	0.28	0.34	0.32
Alaska 4b	UF, A	5.4	0.09	0.18	0.41	<0.02
			0.08	0.18	0.45	<0.02
Alaska	F, A, T	1.7	0.05	0.68	0.65	17
			0.14	0.72	0.78	11

[a]All samples stored frozen except T which was stored at ambient temperature. F = filtered, Fc = filtered after thawing, A = acidified, UF = unfiltered, UA = unacidified. Less than values are AAS detection limits on 100 mL test portions.

in concentration for each of the elements tested; Cd, Mn, Ni and Pb in comparison with these same elements in the Chesapeake Bay sample. These samples were also concentrated from a 100 mL sample volume and can be directly compared not only for concentration but for detection limitation of the GFAAS used in conjunction with the separation procedure.

The concentration and separation proved to be compatible with the GFAAS technique. It enabled greater sensitivity through concentration of the analyte and, by virtue of the separation, the interferences were eliminated, enabling optimization of the instrument for maximum sensitivity and accuracy.

REFERENCES

1. Riley, J. P., and G. Skirrow. Chemical Oceanography. Vol. 1, Academic Press, New York, 1975. pp. 393-394, 416-421.

2. Natelson, S., D. Leighton, and C. Calas. Microchem. J., 4:239, 1962.

3. Riley, J. P., and G. Skirrow. Chemical Oceanography. Vol. III, Academic Press, New York, 1975. pp. 269-408.

4. Davey, E. W., and A. E. Soper. Chapter in Analytical Chemical Methods in Oceanography. T. R. P. Gibb, Jr., ed., Advances in Chemistry Series 147, American Chemical Society, Washington, D.C., 1975. pp.16-21.

5. Mancy, K. H. Instrumental Analysis of Water Pollution Control. Ann Arbor Science Publishers, Inc., Michigan, 1971.

6. Strickland, J. D., and T. R. Parsons. A Practical Handbook of Sea Water Analysis. Fisheries Research Board of Canada, Ottawa, 1972. pp. 111-120.

7. Boyle, E. A., and J. M. Edmond. Chapter in Analytical Chemistry Methods in Oceanography. T. R. P. Gibb, Jr., ed., Advances in Chemistry Series 147, American Chemical Society, Washington, D.C., 1975. pp. 44-55.

8. Morris, A. W. Anal. Chim. Acta, 42:397, 1968.

9. Stolzberg, R. V. Chapter in Analytical Chemistry Methods in Oceanography T. R. P. Gibb, Jr., ed., Advances in Chemistry Series 147, American Chemical Society, Washington, D.C., 1975. pp. 30-43.

10. Blunt, C. W., W. R. Morgan, and D. E. Leyden. Anal. Chim. Acta, 53:463, 1971.

11. Lai, M. G., and H. A. Goya. U.S. Naval Radiological Defense Laboratory, Dept. of Commerce, National Technical Information Service, AD-648485, 1966.

12. Callahan, C. M., J. M. Pascual, and M. G. Lai. U. S. Naval Radiological Defense Laboratory, Dept. of Commerce, National Technical Information Service, AD-647661, 1966.

13. Riley, J. P., and D. Taylor. Anal. Chim. Acta, 40:479, 1968.

14. Riley, J. P., and D. Taylor. Anal. Chim. Acta, 41:175, 1968.

15. Riley, J. P., and D. Taylor. Deep Sea Res., 15:629, 1968.

16. Riley, J. P., and D. Taylor. Deep Sea Res., 19:307, 1972.

17. Knauer, G. A., and J. H. Martin. Limnology and Oceanography, 18:597, 1973.

18. Windom, H. L., and R. G. Smith. Deep Sea Res., 19:727, 1972.

19. Davey, E. W., and A. E. Soper. Limnology and Oceanography, 20:1019, 1975.

20. Chester, R., and J. R. Stoner. Mar. Chem., 2:17, 1974.

21. Abdullah, M. I., and I. G. Royle. Anal. Chim. Acta, 58:283, 1972.

22. Florence, T. M., and G. E. Batley. Talanta, 22:201, 1975.

23. Florence, T. M., and G. E. Batley. Talanta, 23:179, 1976.

24. Morris, A. W. Marine Pollution Bulletin, 5:54, 1974.

25. Chul Lee, Nak Bae Kim, Inn Chong Lee, and Koo Soon Chung, Talanta, 24:241, 1977.

26. Bio·Rad Laboratories. Separating Metals Using Chelex 100 Chelating Resin. Rockville Center, New York, 1976.

27. Bio·Rad Laboratories. Materials for Ion Exchange, Adsorption, Gel Chromatography, Gel Electrophoresis and Hollow Fiber Technique Columns and Stable Isotopes. Rockville Center, New York, 1972.

28. Leyden, D. E., and A. L. Underwood. J. Phys. Chem., 68:2093, 1964.

29. Luttrell, Jr., G. H., C. More, and C. T. Kenner. Anal. Chem., 43:1370, 1971.

30. Holynska, B. Radiochem. Radioanal. Letters, 17:313, 1974.

31. Sides, J. L., and C. T. Kenner. Anal. Chem., 38:797, 1966.

32. VanWilligen, J. H. J. G., and R. C. Schonebaum. Rec. Trav. Chim. Pays-Bas, 85:35, 1966.

33. Campbell, W., E. Spano, and T. Green. Anal. Chem., 38:987, 1966.

34. Campbell, W. J., T. E. Green, and S. L. Law. Amer. Lab., 28, 1970.

35. Van Grieken, R. E., C. M. Bresselers, and B. M. Vanderbraught. Anal. Chem., 49:1326, 1977.

36. Paulsen, P. J. Personal Communications. The National Bureau of Standards, Washington, D.C., 1977.

37. Boumans, P. W. J. M., and F. J. De Boer. Spectrochim. Acta, 31B:355, 1976.

38. Larson, G. F., V. A. Fassell, R. H. Scott, and R. W. Kniseley, Anal. Chem., 47:238, 1975.

39. Larson, G. F., and V. A. Fassel. Anal. Chem., 48:1161, 1976.

40. Forberg, S., and S. Lundgren. Anal. Chem., 23:1202, 1960.

41. Ediger, R. D., F. W. Peterson, and J. D. Kerber. Atomic Absorption Newsletter, 13:61, 1974.

42. Douglas, A. S., and A. Y. Cantillo. Chapter in Analytical Methods in Oceanography. T. R. P. Gibb, Jr., ed., Advances in Chemistry Series 147, American Chemical Society, Washington, D.C., 1975. pp. 56-81.

43. Rains, T. C. Personal Communications. The National Bureau of Standards, Washington, D.C., 1978.

44. Keuhner, E. C., R. Alverez, P. J. Paulsen, and T. J. Murphy, Anal. Chem., 44:2050, 1972.

45. Useller, J. W., NASASP-5074. Office of Technology Utilization. NASA, Washington, D.C., 1969.

46. Dean, J. A., and T. C. Rains. Flame Emission and Atomic Absorption Spectrometry: Components and Techniques, Vol. 2. Marcel Dekker, New York, 1971.

47. Moody, J. R., and R. M. Lindstrom. Anal. Chem., 49:2264, 1977.

48. Moody, J. R., H. L. Rook, P. J. Paulsen, T. C. Rains, I. L. Barnes, and M. S. Epstein. NBS Special Publication 464. W. A. Kirchoff, ed., U.S. Government Printing Office, Washington, D.C., 1977.

49. Maienthal, E. J., and D. A. Becker. NBS Special Technical Note 929. U.S. Government Printing Office, Washington, D.C., 1976.

50. Harrison, S. H., P. D. LaFleur, and W. Zoller. Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis. P. D. LaFleur, ed., U.S. Government Printing Office, Washington, D.C., 1976.

51. The Perkin-Elmer Company. Analytical Methods for Atomic Absorption Spectroscopy. Atomic Absorption Dept., Norwalk, CT, 1973.

52. The Hewlett-Packard Company. Hewlett-Packard Calculator 9830A Plotter PAC for Model 30. Loveland, Colorado, 1974.

53. Kayser, A. C. J. Chem. Educ., 36:128, 1959.

54. Lapp, R. E., and H. L. Andrews. Nuclear Radiation Physics. Prentice-Hall, Inc., 1954. pp. 456-459.

55. Currie, L. A. Personal Communications. The National Bureau of Standards, Washington, D.C., 1978.

56. Meites, L. Handbook of Analytical Chemistry. McGraw-Hill Book Company, New York, 1963. pp. 1-39.

57. Lange, N. A., and G. M. Forker, eds.. Handbook of Chemistry. Handbook Publishers, Inc., Sandusky, Ohio, 1952. p. 1145.

58. Dow Chemical Company. Dowex Chelating Resin A-1, Midland, Michigan, 1959. p. 21.

59. Bleuler, E., and G. J. Goldsmith. Experimental Nucleonics. Holt, Rinehart, and Winston, New York, 1952. p. 236.

60. Friedlander, G., and J. W. Kennedy. Introduction to Radiochemistry. John Wiley and Sons, Inc., New York, 1949. p. 290.

61. Broda, E., and T. Schonfeld. The Technical Application of Radioactivity, Vol. 1, Pergamon Press, New York, 1966. p. 64.

62. Lundell, G. E. F., H. A. Bright, and J. I. Hoffman. Applied Inorganic Analysis: With Special Reference to the Analysis of Metals, Minerals, and Rocks. John Wiley and Sons, Inc., New York, 1953. pp. 133-134.

63. Dean, J. A., and T. C. Rains, ed. Flame Emission and Atomic Absorption Spectrometry: Elements and Matrices, Vol. 3. Marcel Dekker, New York, 1975.

64. Sidgwick, N. V. The Chemical Elements and Their Componds. Oxford University Press, London, England, 1950. pp. 252-254.

APPENDIX A

THE ABUNDANCES OF THE CHEMICAL ELEMENTS IN SEA WATER (1)

ELEMENT	TOTAL CONCENTRATION (MOLAR)	TOTAL CONCENTRATION ($\mu g\ L^{-1}$)
H	55	1.1×10^{8}
He	1.7×10^{-9}	6.8×10^{-3}
Li	2.6×10^{-5}	180
Be	6.3×10^{-10}	5.6×10^{-3}
B	4.1×10^{-4}	4440
C	2.3×10^{-3}	2.8×10^{4}
N	1.07×10^{-2}	1.5×10^{5}
O	55	8.8×10^{8}
F	6.8×10^{5}	1.3×10^{3}
Ne	7×10^{-9}	1.2×10^{-1}
Na	4.68×10^{-1}	10.77×10^{6}
Mg	5.32×10^{2}	12.9×10^{5}
Al	7.4×10^{8}	2
Si	7.1×10^{5}	2×10^{6}
P	2×10^{6}	60
S	2.82×10^{2}	9.05×10^{5}
Cl	5.46×10^{1}	18.8×10^{6}
Ar	1.1×10^{7}	4.3
K	1.02×10^{2}	3.8×10^{5}
Ca	1.02×10^{2}	4.12×10^{5}
Sc	1.3×10^{11}	6×10^{4}
Ti	2×10^{8}	1
V	5×10^{8}	2.5
Cr	5.7×10^{9}	0.3

APPENDIX A (Continued)

ELEMENT	TOTAL CONCENTRATION (MOLAR)	TOTAL CONCENTRATION ($\mu g\ L^{-1}$)
Mn	3.6×10^{-9}	0.2
Fe	3.5×10^{-8}	2
Co	8×10^{-10}	0.05
Ni	2.8×10^{-8}	1.7
Cu	8×10^{-9}	0.5
Zn	7.6×10^{-8}	4.9
Ga	4.3×10^{-10}	0.03
Ge	6.9×10^{-10}	0.05
As	5×10^{-8}	3.7
Se	2.5×10^{-9}	0.2
Br	8.4×10^{-4}	6.7×10^{4}
Kr	2.4×10^{-9}	0.2
Rb	1.4×10^{-6}	120
Sr	9.1×10^{-5}	8×10^{4}
Y	1.5×10^{-11}	1.3×10^{-3}
Zr	3.3×10^{-10}	3×10^{-2}
Nb	1×10^{-10}	1×10^{-2}
Mo	1×10^{-7}	10
Tc		
Ru		
Rh		
Pd		
Ag	4×10^{-10}	0.04
Cd	1×10^{-9}	0.1
In	0.8×10^{-12}	1×10^{-4}
Sn	8.4×10^{-11}	1×10^{-2}
Sb	2×10^{-9}	0.24

APPENDIX A (Continued)

ELEMENT	TOTAL CONCENTRATION (MOLAR)	TOTAL CONCENTRATION ($\mu g\ L^{-1}$)
Te		
I	5×10^{-7}	60
Xe	3.8×10^{-10}	5×10^{-2}
Cs	3×10^{-9}	0.4
Ba	1.5×10^{-7}	2
La	2×10^{-11}	3×10^{-3}
Ce	1×10^{-10}	1×10^{-3}
Pr	4×10^{-12}	6×10^{-4}
Nd	1.9×10^{-11}	3×10^{-3}
Pm		
Sm	3×10^{-12}	0.5×10^{-4}
Eu	9×10^{-13}	0.1×10^{-4}
Gd	4×10^{-12}	7×10^{-4}
Tb	9×10^{-13}	1×10^{-4}
Dy	6×10^{-12}	9×10^{-4}
Ho	1×10^{-12}	2×10^{-4}
Er	4×10^{-12}	8×10^{-4}
Tm	8×10^{-13}	2×10^{-4}
Yb	5×10^{-12}	8×10^{-4}
Lu	9×10^{-13}	2×10^{-4}
Hf	4×10^{-11}	7×10^{-3}
Ta	1×10^{-11}	2×10^{-3}
W	5×10^{-10}	0.1
Re	2×10^{-11}	4×10^{-3}
Os		
Ir		
Pt		
Au	2×10^{-11}	4×10^{-3}

APPENDIX A (Continued)

ELEMENT	TOTAL CONCENTRATION (MOLAR)	TOTAL CONCENTRATION ($\mu g\ L^{-1}$)
Hg	1.5×10^{-10}	3×10^{-2}
Tl	5×10^{-11}	1×10^{-2}
Pb	2×10^{-10}	3×10^{-2}
Bi	1×10^{-10}	2×10^{-2}
Po		
At		
Rn	2.7×10^{-21}	6×10^{-13}
Fr		
Ra	3×10^{-16}	7×10^{-8}
Ac		
Th	4×10^{-11}	1×10^{-2}
Pa	2×10^{-16}	5×10^{-8}
U	1.4×10^{-8}	3.2

APPENDIX B

THE SELECTIVITY COEFFICIENTS FOR CHELEX 100 RESIN BASED ON Zn^{+2} (26)

IONIC SPECIFIC	SELECTIVITY COEFFICIENT
Hg^{+2}	1060
Cu^{+2}	126
UO^{+2}	5.70
Ni^{+2}	4.40
Pb^{+2}	3.88
Zn^{+2}	1.000
Co^{+2}	0.615
Cd^{+2}	0.390
Fe^{+2}	0.130
Mn^{+2}	0.024
Ba^{+2}	0.016
Ca^{+2}	0.013
Sr^{+2}	0.013
Mg^{+2}	0.009
Na^{+1}	0.000,000,1

APPENDIX C

TYPICAL SUB-BOILING DISTILLED WATER IMPURITY CONCENTRATIONS PPB BY WT (ng/g) (44)

ELEMENT	WATER FROM SUB-BOILING STILL
Pb	0.008
Tl	0.01
Ba	0.01
Te	0.004
Sn	0.02
Cd	0.005
Ag	0.002
Sr	0.002
Zn	0.04
Cu	0.01
Ni	0.02
Fe	0.05
Cr	0.02
Ca	0.08
K	0.09
Mg	0.09
Na	0.06
	Σ 0.5 ppb

APPENDIX C (Continued)

TYPICAL SUB-BOILING DISTILLED NITRIC ACID
IMPURITY CONCENTRATIONS PPB BY WT (ng/g) (44)

ELEMENT	ACID FROM SUB-BOILING STILL
Pb	0.02
Tl	--
Ba	0.01
Te	0.01
Sn	0.01
In	0.01
Cd	0.01
Ag	0.1
Sr	0.01
Se	0.09
Zn	0.04
Cu	0.04
Ni	0.05
Fe	0.3
Cr	0.05
Ca	0.2
K	0.2
Mg	0.1
Na	1
	Σ 2.3 ppb

APPENDIX D

THE EXPERIMENTAL CONDITIONS FOR ANALYSIS OF THE 2.5 M HNO_3 FRACTION CONTAINING THE TRACE METALS USING A PERKIN-ELMER MODEL 603 ATOMIC ABSORPTION SPECTROPHOTOMETER EQUIPPED WITH A HEATED GRAPHITE ATOMIZER, HGA-2100

		P & E 603		HGA-2100			
Element	Wavelength nm	SBW nm	Scale Expansion	Drying T-sec	Charring T-sec	Atomization T-sec	Gas
Cd	228.8	0.7	1	100-30	200-20	2100-7	Ar[a]
Co	240.7	0.2	2	100-30	500-30	2700-7	Ar[a]
Cu	324.7	0.7	1	100-40	700-30	2500-6	Ar[a]
Fe	248.3	0.2	2	100-30	600-30	2700-7	Ar[a]
Mn	279.5	0.7	2	100-30	300-30	2700-7	Ar[a]
Ni	232.0	0.7	5	100-30	1000-30	2700-6	Ar[a]
Pb	283.3	0.7	3	100-40	400-30	2200-7	Ar[b]
Zn	213.9	0.7	0.5	100-30	500-20	2000-7	Ar[b]

[a]Interrupt mode.

[b]Normal mode.

APPENDIX E

A LIST OF RESIN VOLUME FOR CHELEX 100 IN DIFFERENT IONIC FORMS BASED ON Na AS 1.00 (58)

Resin volume in different ionic forms	
Na^{+}	1.00
H^{+}	0.45
Fe^{++}	0.45
Zn^{++}	0.55
Ca^{++}	0.53
K^{+}	1.06
Li^{+}	0.98
Ag^{+}	0.70

APPENDIX F

A CRYSTAL RESIDUE OBSERVED IN FROZEN NEUTRAL SEA WATER SAMPLES

While analyzing the NBS and Alaskan samples, a sample inhomogeneity was observed. Some samples which had been unacidified and frozen had white crystals at the bottom of the container when thawed. Shaking and heating did not aid in redissolving these crystals and they did not appear in the moderate to strongly acidified samples.

A sample of these crystals was extracted and analyzed by spark source mass spectrometry. They were found to contain Ca almost exclusively. The crystals produced a violent evolution of gas upon contact with (1+1) HNO_3.

Several samples of unacidified sea water were evaporated to dryness. Distilled water of equal volume was heated to reduce the CO_2 gas dissolved in the water. The evaporated salts were mixed with the distilled water and a crystal residue similar to the NBS and Alaskan samples resulted.

Using the data obtained, the crystals were hypothesized to be $CaCO_3$ compounds in various forms of hydration. The formation of these crystals was suspected to originate in both the frozen and the evaporated sea water by this mechanism:

$$Ca(HCO_3) \longrightarrow CaCO_3\downarrow + H_2O + CO_2\uparrow.$$

The calcium carbonate is much less soluble in water in the absence of carbon dioxide (64). Although acid was found able to put the crystals into solution, an alternative method was tested where the previous equilibrium was reversed:

$$CaCO_3 + H_2O + CO_2 \longrightarrow Ca(HCO_3)_2.$$

Two methods of redissolving these crystals in their original solutions were successful. First, a 2 cm^3 piece of dry ice was sufficient to place the crystals back into solution in a 1 liter sea water sample from which they had been frozen out. The time required was thirty to sixty minutes. Secondly, the sample was placed in an ice bath and a positive pressure of CO_2 filtered gas was applied for twenty-four to thirty-six hours, which totally redissolved the crystals in a 1 liter sample.

The only sample analyzed which contained any crystals was Alaska 3a. The sample was decanted into the three 100 mL samples necessary for analysis, leaving the crystals behind. Sample Alaska 1b also exhibited the crystals when thawed, but they dissolved slowly upon standing, perhaps due to the higher acid content.

The technique developed for redissolving the crystals in neutral sea water samples was not used on samples Alaska 3a and 1b. Further study was desired on the possibility of contamination from dry ice, and adsorption by the container

walls of warm, mobile, unacidified samples was a possibility (48). The remainder of the samples exhibiting the "crystal freeze out" phenomenon were duplicate samples and analysis was unnecessary.